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Aneosinophilia in a Patient with Bronchial Asthma

By

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Eosinophilia is a frequent finding in untreated patients with bronchial asthma. This paper deals with the report of a case of this disease where, surprisingly no eosinophilic leukocytes were found on repeated examination of the peripheral blood. Moreover no eosinophils could be demonstrated in representative specimens from the patient's sputum, in a bone marrow smear or in various organs obtained at autopsy The conclusion was reached that the underlying mechanism was probably a primary inability to produce leukocytes of the eosinophilic series.

Case report

71-year-old woman whose previous history revealed nothing of particular interest. Since the age of 20 she had suffered from an overt bronchial asthma. The first admission to Falu hospital occurred five months before death because of status asthmaticus. Physical examination of the heart was normal. Blood pressure on admission was 170/110 mm Hg and thereafter about 140/80 mm Hg An electrocardiogram revealed signs of hypertrophic left chamber Hemoglobin and R. B. C. were normal. The white-cell count ranged 3,500—6,000/mm Differential count of W B. C. on admission neutrophils 72, eosinophils 0, basophils 0 lymphocytes 19 and monocytes 9 per cent. As to further examinations of the number of eosinophils — see below Sedimentation rate ranged 6 to 15 mm. There was no albumin in the urine N P N was 32 mg per 100 ml. Microscopic examination of the urine was normal. She improved markedly after treatment with aminophylline and isoprotenerol and was discharged after four weeks, when she felt relatively well.

She was re-admitted to the hospital ten days later because of a new attack of status asthmaticus. As earlier hemoglobin, red-cell and white-cell count were normal. Differential count of W B C. on admission Neutrophils 53 eosinophils 0 basophils 0 lymphocytes 44 and monocytes 3 per cent. Serum protein was 7 9 per cent. Electrophoretic examination of serum albumin 4.55, α_1 0.43, α_2 0.72, β 0.84 and γ 1 56 g per 100 ml. Thymol extinction 0.06. Takata normal. Alkaline phosphatase 7 4 Bodansky units. Puncture of the sternal bone marrow was performed when there were no symptoms or physical findings of asthma. The specimen revealed a normal distribution of the cells with the exception that no eosinophils or eosinophilic myelocytes were found. The method described by Mårtensson and Vikbladh (3) to demonstrate leukocyt agglutinating factor was adopted to show whether or not antibodies against the eosinophils were present in the patient serum. The results were negative.

She was treated in the hospital among others with prednisolone 5 mg four times

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daily for 11 days. She improved gradually and her general condition was good when she left the hospital after 3 weeks stay

Final admission to the hospital occurred two months later. She entered the hospital because of severe attacks of asthma during the previous two weeks and drowsiness that had lasted 24 hours. Her general condition on admission was poor. She did not react when spoken to. There was a moderate cyanosis of her lips. She revealed signs of an incipient pulmonary edema. She deteriorated gradually and died on her fourth day in hospital.

Throughout the time of observation no eosinophils were found in her peripheral blood. Thus, during 5 months, 31 determinations with the use of Rud's method (5) were made on various days and in all of these instances no eosinophils were demonstrated. The examinations were mostly performed in the hospital but also on three occasions when she was in her home and had no troubles at all without medication. Eosinophilic counts were made on three occasions when she was under treatment with prednisolone or adrenaline. No eosinophils were found in her sputum. As mentioned above, no eosinophils or eosinophilic myelocytes were found in a bone marrow smear

At autopsy adhesions of the pleura, emphysema and signs of bronchitis were found. The wall of the left chamber was hypertrophic with old scars, probably signs of a previous myocardial infarction. There was a moderate sclerosis of the coronary arteries, aorta and the pulmonary artery. Examination of the liver and spleen revealed signs of congestion. Some ten gallstones were found in the gall-bladder. There was a moderate atrophy of the cortex of the kidneys. The uterus and ovaries were likewise atrophic. The pancreas, brain, hypophysis and adrenal glands were macroscopically normal.

The microscopic examination of various organs was made by professor Ringertz (Sabbatsbergs sjukhus, Stockholm). Specimens of the hypophysis and adrenal glands showed a normal picture. There were scattered inflammatory changes in the lungs mostly with lymphocytes but also with some neutrophils and plasma cells but nowhere with any eosinophils. The neutrophils were abundant in the liver sinusoids but no eosinophils were found. Furthermore, no eosinophils were found in the spleen. Professor Ringertz arrived at the following conclusion: The result of the microscopic examination thus revealed pulmonary findings typical of bronchial asthma and death during a period of frequent attacks of asthma. The lack of eosinophilic leukocytes in inflammatory patches is, however, remarkably divergent from the picture usually seen. There was venous congestion of the liver and spleen which seems to have been caused by a failure of the heart.

Eosinophils in blood specimens obtained from relatives of the patient.

The patient's parents had been cousins. 24 of their descendants were still living and 21 of them were examined for the number of eosinophils in the peripheral blood. The patient had no brothers but one sister who was still alive; the blood specimen obtained from her sister revealed 350 eosinophils per mm³. There were only three descendants of the patient herself (all grandchildren) and all showing a fairly low number of eosinophils, or 80, 40 and 20 per mm³ respectively. Three relatives with allergic diseases had eosinophilia. The remaining relatives who were examined presented eosinophils within the normal range

Discussion

The most remarkable observation made on this patient with bronchial asthma was that no circulating eosinophils were found on 31 determinations made during a period of 5 months. The specimens were obtained during periods of asthma as well as during symptom free periods. The patient was not treated with ACTH and only on three occasions with prednisolone. Furthermore eosinophils or their precursors were absent in a bone-marrow aspirate and no eosinophils were found on microscopic examination of her sputum and of various organs obtained post mortem.

Eosinophilia is a main finding in untreated patients with bronchial asthma

particularly between the attacks. However not infrequently a value of circulating eosinophils within the normal range is encountered (1). Therefore, it seemed justifiable to compare the results obtained in our patient with the number of eosinophils in normal persons as recorded in the literature. The question was to calculate the probability of finding no eosinophils in a normal person on 31 consequetive determinations. Most likely then, this probability is not higher in a patient with bronchial asthma.

The series of normal persons published by Rud (5) was chosen for this purpose. Using logarithmic analysis (4) of this material the probability of finding no circulating eosinophils in 31 consecutive counts in normal persons was shown to be about 1 in 25^{14} millions. The probability that the observed values of the patient's eosinophils are still within the normal values can thus be regarded as non-existent from a practical point of view. Therefore, other causes of this finding have to be discussed.

The possibility of an existing hyperfunction of the hypophysis or of the adrenal glands can also be ruled out in this case. The patient did not show the clinical picture of a Cushing' syndrome and there were no macroscopical or microscopical aberrations from the normal picture to be found in the hypophysis or in the adrenal glands.

It is evident from our observations that the lack of eosinophils cannot possibly be explained by the existence of an immunological factor selectively directed against this kind of leukocyte.

In the discussion of the underlying mechanism of the absence of eosinophils as observed, the following question arises: was it possible that the patient had previously had a normal or even high number of eosinophils in the bone marrow and in the blood? If so, the course of the disease might have been analogous to that found in aplastic crises of hemolytic anemias or in advanced cases of agranulocytosis with hypocellular bone marrow. However, this seems to be a most speculative hypothesis. Sufficient to mention there has been no report of such a selective aplasia of the eosinophilic series in the overwhelming literature of allergic disorders with the possible exception of one case, although no such an etiologic mechanism was suggested in the report (2). That patient, a 28-year-old woman showed certain similarities with the one described in the present paper, having an allergic disorder (urticaria and drug fever) and being without circulating eosinophils on repeated examinations.

Various causes of the absence of eosinophils observed in this case can therefore most likely be ruled out. There remains thus the possibility of a primary inability to produce leukocytes belonging to the eosinophilic series. The investigation of the patient's family disclosed that her parents had been cousins and that her three descendants still alive all had a low number of eosinophils in the peripheral blood on examination. The possible relationship between the lack of eosinophils as observed and her asthma is obscure, since so little is known regarding the function of the eosinophils. In this connection it is, however, interesting to draw attention to the fact that the only published case of the same sort (2) also had an allergic disease.

Summary

A case report is presented of a woman, aged 74, with overt bronchial asthma, showing a lack of eosinophils in her peripheral blood on 31 determinations

during a period of 5 months Furthermore, there were no eosinophils to be found in sputum or in a specimen obtained at bone marrow puncture. This observation was not due to the treatment, *i e.* steroids or adrenaline At autopsy no eosinophils were found in various organs including the lungs, which was surprising since she died from repeated severe asthmatic attacks.

The primary defect in this patient was probably a failure by the bone marrow to produce eosinophilic leukocytes.

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Studies on the Clearing Phenomenon in Essential Hyperlipemia

By

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The rôle played by intravascular clearing in the physiological removal of triglycerides from the blood stream is somewhat uncertain and probably of minor significance. I travenous heparin produces, however both in normal subjects and in most cases of essential hyper lipemia a considerable reduction of the serum lipids (Lever et al. 1954 and 1955 Kuo et al. 1956, Schettler et al 1958)

In assessing the clearing-factor activity of plasma *in vitro* after intravenous doses of 100 and 5 mg of heparin respectively Hood et al. (1954) and Angervall nd Hood (1957) found no difference n the post heparin clearing factor activity in plasma in cases of myocardial infarction and essen tial hypercholesterolemia as compared with normals. In a case of essential hyperlipemia, after intravenous injection of heparin (5 mg) Joyner (1955) found *in vivo* clearing of the same magnitude as that found by Block, Barker and Mann (1951) in male atherosclerotic subjects 3 hours after fat meal. Anfinsen (1955) and Havel and Gordon (1960) reported, how ever a family with hyperlipemia and with peculiar clinical features, with only traces of lipoprotein lipase after a large dose of heparin intravenously Intense heparin administration failed to reduce the patients lipoprotein and triglyceride concentrations significantly

However Klein, Lever and Fekete (1959) stated that while clearing activity was less in the hyperlipemic patient the production of clearing factor was actually demonstrably larger than in normals after heparin intravenously These authors have also, in a number of papers (1957 1959) described the occurrence of heat labile inhibitory factors in essential hyperlipemia, active towards both post heparin lipoprotein lipase and pancreatic lipase.

The above authors also considered their labile inhibitors to be different from the heat-stable inhibitors (probably of a mucoprotein nature) described as occur ring in normal plasma by Hollet and Meng Hood and Angervall (1957) found a relation between inhibition of clearing and the level of cholesterol in Cohn frac tion I + II + III

Of factors playing a rôle in the clearing phenomenon the following (among others) might be considered

a) The amount of lipoprotein lipase

b) The ability of chylomicra to be cleared.

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Table I. Essential hyperlipemia. Optical density and FFA in plasma after 3 mg of heparin intravenously

Case		P	P	P_{10}	P_{15}
S. Ö	OD	1.25	0.58	0.79	0.86
4.4.57	FFA	—	—	—	—
S. Ö	OD	5 × 1.05	5 × 1.27	5 × 1.47	5 × 1.47
3.2.58	FFA	0.61	—	1.00	1.13
E. A.	OD	0.50	0.59	0.72	1.01
6.12.57	FFA	0.70	0. 8	0.81	0.81
E. A.	OD	0.70	0.75	0.77	0.81
9.12.57	FFA	0.86	1.70	1.73	1.58
H. K.	OD	1.16	1.035	—	0.98
5.6.58	FFA	0.46	0.45	—	0.85
O. W.	OD	0.18	0.17	0.14	0.15
1.8.58	FFA	0.35	0.50	0.53	0.59

P = plasma before heparin injection.
P_5, P_{10}, P_{15} = plasma 5, 10 and 15 minutes after 3 mg of heparin intravenously.
OD = optical density.
FFA = free fatty acids in mEq/L.

c) Inhibitors of the lipase in plasma.
d) Fatty-acid acceptor capacity.

The last mentioned factor may be assessed in a simplified manner as discussed below. The cases of essential hyperlipemia in this report have been studied more and less completely in all the above respects.

Material

The clinical material consisted of 7 cases of essential hyperlipemia. Care was taken not to include cases with recent cardiovascular catastrophes or signs of infection. For purposes of comparison a group of 16 healthy students or blood donors was used. In one of the studies of plasma inhibitors of clearing age and sex matched controls were used.

Methods

Plasma

Blood was drawn in the fasting state in the morning in prechilled, oxalated, siliconized bottles, and centrifuged at 0° C at 800 g for 20 minutes for sedimentation of blood cells. If plasma free from chylomicra was needed another centrifugation at 0° C at 25 000 g for 1 hour was performed and the layer under the creamy supernatant was collected. The plasma was then immediately used for the experiments.

Serum lipid determinations

Total serum cholesterol was determined according to Cramer and Isaksson (1959) and total fat gravimetrically on the same ethanol-acetone extract as follows. After evaporation of an aliquot the residue was re-extracted with chloroform and an aliquot thereof evaporated and weighed. Standard deviation for 75 duplicate total fat determinations was 57 mg per cent.

Triglyceride was determined as glyceride-glycerol according to the modified (Carlson 1959) method of Carlson and Wadström (1959).

Fat tolerance test

The fat tolerance test was performed in the fasting state in the morning, blood samples being drawn before and 4 hours after 200 ml (= 80 g butter fat) heavy cream was given.

Chylomicra

Lipemic plasma was centrifuged in the cold at 25 000 g for 1 hour. The creamy top layer was collected and recentrifuged under the same conditions in physiological saline and the new top layer was suspended in physiological saline.

In vitro clearing systems

The reaction was performed at 24° C and was done either by measuring the decrease in optical density at 650 $\mu\mu$ in a Beckman model B spectrophotometer in 1 cm cuvettes or by measuring the release of free fatty acids (= FFA). Early in the investigation this was done according to Grossman et al. (1954) and later according to Svanborg and Svennerholm (1958). In the method of Grossman et al. (1954) a microburette described by Heatley (1935) and modified by Løvtrup and Roos (1959) was used. The standard deviation of 70 duplicate determinations was ± 0.035 mEq/L.

System I: 1 part post-heparin plasma.
1 » substrate emulsion.

System II 0.2 parts post-heparin plasma.
0.3 » substrate emulsion.
4.5 plasma.

Post-heparin plasma

In the studies where the lipase activity after injection of heparin was assayed, post-heparin plasma from the subject to be tested was used. In the studies where inhibition of the enzyme was studied and in the experiments with chylomicra as substrate standardized source of enzyme was obtained by drawing blood from normal donors 60 minutes after the injection of 100 mg of heparin intravenously. These plasmas were mixed, divided into 2 ml portions and stored deep frozen. The activity of this standard post-heparin plasma was fairly stable as judged by tests throughout the experimental series in system II containing inhibitor-freed plasma.

Substrate emulsion

As earlier described (Angervall and Hood 1957) homogenized cream was used as substrate diluted in physiological saline to a constant initial optical density in both the systems.

Plasma in system II

This plasma was freed either from chylomicra or from inhibitors as described by Angervall and Hood (1957)

System I was used for the assay of lipoprotein lipase activity after the injection of heparin. It was also used in the studies of chylomicra as substrate for the enzyme.

System II was used for the assay of lipoprotein lipase activity after the injection of heparin, in the milieu of inhibitor-freed plasma. This system was also used in the studies of plasma inhibitors where the chylomicron-freed plasma from the patient to be tested was compared with plasma from normal control and with an inhibitor-freed plasma.

Results

Lipoprotein lipase activity after injection of heparin

I vivo experiments

In 6 hyperlipemics plasma was sampled 5 10 and 15 minutes after injection of 3 mg of heparin intravenously (see

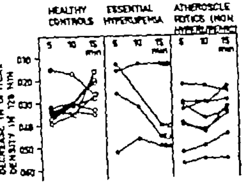


Fig. 1 Blood samples drawn 5, 10 and 15 minutes after 3 mg of heparin intravenously Plasma from hyperlipemics centrifuged at 25,000 *g* for one hour and substrate tested. Clearing activity measured as decrease in optical density in 120 minutes. Healthy controls and nonhyperlipemic atherosclerotics are from table I Angervall and Hood 1957

table I) A decrease in the optical density of plasma was found in 3 of these patients, to a marked extent only in one. In the other 3 cases there was an increase of optical density When however the plasma was tested *in vitro* in 4 of the same experiments, the lipoprotein lipase activity was as good as, or better than in normals (see below) This means that the *in vitro* assay system can show lipase activity even when this is not detectable by a decreased density *in vivo*

The lipoprotein lipase activity was also measured by the release of FFA *in vivo* In all cases there was an increased amount of FFA in plasma 5 10 and 15 minutes after the injection of heparin. This occurred whether there was an increase or a decrease in optical density

In vitro experiments

When the post-heparin clearing activity was assayed in system I, the decrease in optical density seemed as good as in normals in 3 of 4 hyperlipemics (fig 1) The activity seemed to increase from 5

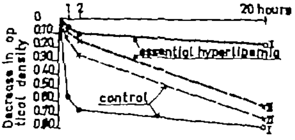


Fig 2 In vitro clearing t 24 C. P_{Hep} 100 mg. 60′ centrifuged 25 000 g 90′ at 0° C.
System I: 2 ml P_{Hep} + 2 ml cream NaCl.
System II 0.2 ml P_{Hep} + 0.3 ml cream N Cl + 4.5 ml P DP (inhibitor freed plasma) Initial optical density 1 35

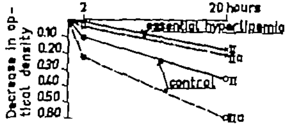


Fig 3 Inhibition towards clearing in plasma. System II 0.2 ml P_{Hep} bank + 0 3 ml cream NaCl + 4.5 ml P 25,000 g
System IIa 0.2 ml P_{Hep} bank + 0.3 ml cream NaCl + 0 6 ml P 25,000 g + 3.9 ml NaCl. System IIa was a check that the fatty acid acceptor was not a limiting factor For an explanation of the increased clearing in this system, see Angervall and Hood 1957 fig 1

Table II Inhibition of in vitro clearing by plasma from essential hyperlipemics and normals as compared with inhibitor freed plasma

	Decrease in optical density in 20 hours			Inhibition in %	
	Essential hyperlipemia	Controls[1]	P_0DP	Essential hyperlipemia	Controls
S. Ö.					
Ad libitum diet	0.18	0.35	0.60	71	42
Fat poor diet	0.35	0 44	0.64	45	31
E. A.					
Ad libitum diet	0.15	0.29	0.53	71	45
Fat-poor diet	0.30	0.26	0.58	48	55
H. C.					
Ad libitum diet	0.19	0.31	0.54	66	42

All controls on *ad libitum* diet.

Clearing according to system II Plasma from essential hyperlipemics was centrifuged at 25,000 g for one hour and the almost clear subnatant was tested. The difference of clearing in pooled inhibitor-freed plasma /P_0DP/ and the plasma to be tested, expressed as % of clearing in inhibitor freed plasma.

to 15 minutes after the injection, while the opposite occurred in normals. Also the activity at 15 minutes seemed larger in 3 of the hyperlipemics and 2 of the atherosclerotics than that obtained at any time after injection in any of the normals. In the fourth case of hyperlipemia (S Ö) the decrease in optical density was consistently low 5 10 and 15 minutes after injection. In another experiment in this patient there was no release of FFA. These observations led to a closer analysis of this patient and the following experiments were performed.

Blood drawn 60 minutes after 100 mg of heparin intravenously was lipemic and had to be centrifuged at 25 000 g for 90 minutes at 0 The almost clear subnatant was tested for lipoprotein lipase activity (see fig 2) In system I there was only a small decrease in optical density in the patient's plasma as compared with a post heparin plasma used in the control In system II the lipoprotein lipase concentration was 12 5 times less than that in system I In spite of this the patient's post heparin plasma in system II produced a greater decrease in optical density than in system I The reason for this difference must be that the plasma milieu in system II was inhibitor-freed plasma.

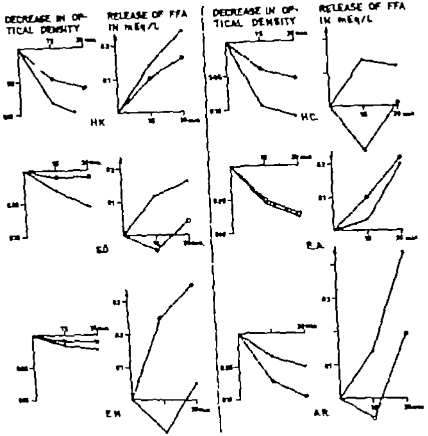


Fig. 4. Plasma as ... milieu for lipoprotein lipase. Decrease in optical density in System II fig. 5. Release of FFA except for the higher substrate concentration this system was of the same pattern. •—• hyperlipemics. ○—○ sex and age matched controls.

The difference in decrease in optical density between the patient's plasma and the control plasma in system II was small, i. e. there was almost as much lipoprotein lipase activity in the patient's plasma as in the normal control plasma. Another similar experiment with the same patient's plasma in system I showed an increase in optical density of 0.18 after 2 hours, when the control plasma decreased by 0.77

At 2 different times FFA were determined in the same patient's plasma 1 hour after 100 mg of heparin intravenously the values being 3.38 and 3.20 mEq/l respectively. If all FFA were assumed to be bound to albumin, there would have been about 80 % saturation of the albumin (patient's serum albumin was 4.2 % or 0.6 mEq/l plasma. one mole albumin can bind 7—8 moles of fatty acids). This might be one possible explanation for the small or absent clearing activity in this heparin plasma when tested in system I.

Plasma inhibitors

This problem was investigated in two series. In the first series the optical clearing *in vitro* was measured in system

Table III In vitro clearing of chylomicra from controls obtained 4 hours after 80 g of butter fat

Exp.	Case	Decrease in optical density			
		P_{Hep} 12.5		P_{Hep} 25	
		2 hrs.	20 hrs.	2 hrs.	20 hrs.
1	S. Ö (fasting)	0.12	0.54	0.45	0.70
	Control	0.25	1 13	0.70	1 19
2	S. Ö (fasting) fresh chyl.	0.24	0.76	0.53	0.75
	S. Ö (fasting) old chyl.	0.18	0.62	0.47	0.72
	Control	0.50	1 09	0.76	1 06
3	H. C. (fasting)	—	—	0.15	0.71
	Control AP	—	—	0.39	0.96
	Control JE	—	—	0.50	0.98
4	E. A. (fasting)	—	—	0.05	0.19
	E. A. post alimentary	—	—	0.22	0.45
	Control CFG	—	—	0.37	0.66
	Control G	—	—	0.53	0.63

P_{Hep} 12.5 = 1 part post-heparin plasma + 7 parts fasting plasma from normals.

P_{Hep} 25 = 1 part post-heparin plasma + 3 parts fasting plasma from normals.

II where 90 % of the reaction mixture consisted of the plasma to be studied. FFA were not followed The concentrations of substrate and enzyme (standard post heparin plasma) were kept constant The lipemic plasma of the case of essential hyperlipemia to be tested for inhibitors was freed from chylomicra. Plasmas from essential hyperlipemia and from normal blood donors were tested parallely and a reaction system with a standard inhibitor freed plasma was also included The difference in clearing between the system with inhibitor freed plasma and that with the plasma to be tested was expressed as per cent of the clearing reached in inhibitor freed plasma. Fig 3 represents a typical experiment. System IIa was a check that the fatty acid acceptor capacity was not a limiting factor The results are listed in table II

In the 3 cases of essential hyperlipemia tested, there was much greater inhibition than in the controls. In 2 of these the same experiment was also performed on a fat poor diet. In both cases there seemed to be an increased inhibition when on *ad libitum* diet. However these last two studies might have been invalidated because the substrate concentration was not kept equal in experimental and control systems.

In another series the same types of experiment were performed but now with separate use both of FFA and of optical clearing as parameters. In this series age and sex matched controls were used. The controls had normal serum lipid levels. In the system where FFA was followed the concentration of the substrate was kept above its rate limiting level for the enzyme concentration in question. This was demonstrated by the fact that a linear relationship between FFA release and time was found in preliminary experiments indicating a zero order reaction during at least 30 minutes. In this way a higher neutral fat content still remaining in the plasma centrifuged at 25 000 *g* from the cases of essential hyperlipemia should have no influence in respect of the concentration of the substrate Except for the higher substrate concentration this system was as for system II Fig 4 demonstrates the results. It is seen that these are variable Four out of 6 cases of hyperlipemia showed less FFA release than the controls and 5 out of 6 less optical clearing

Chylomicra as a substrate for clearing Washed chylomicra from essential hyperlipemia, collected in the fasting state were compared with chylomicra of nor

Table IV Cholesterol and total fat contents of chylomicra in essential hyperlipemia

Cases	Cholesterol as mg % in plasma centrifuged		Diff.	Total fat as mg % in plasma centrifuged		Diff.	Cholesterol as % of total fat in chylomicra
	800 g	25,000 g		800 g	25,000 g		
S. O. 11. 3.57	590	236	234	3,620	1,540	2,080	12.4
25. 6.57	482	226	236	4,400	2,150	2,250	11.4
16. 8.57	241	176	65	2,090	1 410	680	9.6
20. 8.57	314	229	85	2,600	1,820	780	10.9
23. 1.58	509	273	236	3,860	1,720	2,140	11.2
$H_{100, 60}$ 27. 6.57	350	293	57	1,840	1,630	210	27.0
$H_{100, 60}$ 16. 8.57	227	197	30	1,560	1 410	150	20.0
$H_{100, 60}$ 28. 8.57	293	262	33	1,990	1,860	130	25.8
H. C. 8.10.57	474	363	111	2,960	1,620	1,340	8.0
28.11.57	488	440	48	2,480	2 135	345	13.5
6. 2.58	493	301	194	2,305	1 145	1 160	16.7
F 6. 2.58	462	294	168	2,620	1,655	963	17.4
5. 6.58	470	393	77	2,710	1 775	933	8.2
$H_{5, 15}$ 5. 6.58	448	340	108	2,080	1,520	560	18.7
E. A. 19.11.58	410	370	40	1,930	1,685	245	16.3
21.11.58	447	407	40	1,930	1 720	210	19.0

Cholesterol and total fat as mg in plasma centrifuged at 800 g for 20' and in the subnatant of plasma centrifuged at 25,000 g, for 60' The ratio

$$\frac{\text{Cholesterol}_{800} - \text{Cholesterol}_{25,000}}{\text{Total fat}_{800} - \text{Total fat}_{25,000}} \quad 100$$

the content of cholesterol in chylomicrons as of the total fat in chylomicrons.

F = samples drawn 4 hrs after fat meal of 80 g butter fat.
$H_{100, 60}$ = samples drawn 60 min. after 100 mg of heparin intravenously
$H_{5, 15}$ = samples drawn 15 min. [illegible] 5 mg of heparin intravenously

mals, obtained 4 hours after 200 ml heavy cream had been given in regard to their ability to act as a substrate for the lipase In these experiments system I was used. The results are shown in table III The chylomicra from subjects with essential hyperlipemia generally cleared only half as fast as alimentary chylomicra from normals under the same experimental conditions. After 20 hours the hydrolytic and synthetic reactions catalyzed by the enzyme should have reached equilibrium (according to the studies of Borgström and Carlson 1957) The decrease in optical density would therefore seem to have reached its maximum before this time. It is evident that the chylomicra from essential hyperlipemia cannot be cleared as much as the alimentary chylomicra from normals.

In the patient S O., chylomicra obtained on two different days were cleared at about the same rate, and at a lower rate than in the controls.

Table III In vitro clearing of chylomicra from controls obtained 4 hours after 80 g of butter fat

Exp.	Case	Decrease in optical density			
		PHep 12.5		PHep 25	
		2 hrs.	20 hrs.	2 hrs.	20 hrs.
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	Control	0.50	1 09	0.76	1 06
3	H. C. (fasting)	—	—	0.15	0.71
	Control AP	—	—	0.59	0.96
	Control JE	—	—	0.50	0.98
4	E. A. (fasting)	—	—	0.05	0.19
	E. A. post-alimentary	—	—	0.22	0.45
	Control CFG	—	—	0.57	0.66
	Control G	—	—	0.53	0.63

PHep 12.5 = 1 part post-heparin plasma + 7 parts fasting plasma from normals.

PHep 25 = 1 part post-heparin plasma + 3 parts fasting plasma from normals.

II where 90 % of the reaction mixture consisted of the plasma to be studied. FFA were not followed. The concentrations of substrate and enzyme (standard post heparin plasma) were kept constant The lipemic plasma of the case of essential hyperlipemia to be tested for inhibitors was freed from chylomicra. Plasmas from essential hyperlipemia and from normal blood donors were tested parallely and a reaction system with a standard inhibitor freed plasma was also included The difference in clearing between the system with inhibitor freed plasma and that with the plasma to be tested was expressed as per cent of the clearing reached in inhibitor freed plasma. Fig 3 represents a typical experiment. System IIa was a check that the fatty-acid acceptor capacity was not a limiting factor The results are listed in table II

In the 3 cases of essential hyperlipemias tested there was much greater inhibition than in the controls. In 2 of these the same experiment was also performed on a fat poor diet In both cases there seemed to be an increased inhibition when on *ad libitum* diet However these last two studies might have been invalidated because the substrate concentration was not kept equal in experimental and control systems.

In another series the same types of experiment were performed but now with separate use both of FFA and of optical clearing as parameters. In this series age- and sex matched controls were used. The controls had normal serum lipid levels. In the system where FFA was followed the concentration of the substrate was kept above its rate limiting level for the enzyme concentration in question. This was demonstrated by the fact that a linear relationship between FFA release and time was found in preliminary experiments, indicating a zero order reaction during at least 30 minutes In this way a higher neutral fat content still remaining in the plasma centrifuged at 25 000 *g* from the cases of essential hyperlipemia should have no influence in respect of the concentration of the substrate Except for the higher substrate concentration this system was as for system II Fig 4 demonstrates the results It is seen that these are variable Four out of 6 cases of hyperlipemia showed less FFA release than the controls and 5 out of 6 less optical clearing

Chylomicra as a substrate for clearing Washed chylomicra from essential hyperlipemia collected in the fasting state were compared with chylomicra of nor

open to criticism. Earlier studies of our own have shown the necessity of having the plasma under investigation constitute close to 100 % of the reaction mixture. When comparing plasma from an essential hyperlipemia patient to plasma from a normal, one meets the difficulty that even a plasma spun for hours at 25,000 *g* contains more lipid in the cases of essential hyperlipemia. In other words, there is more substrate present for the lipoprotein lipase. It was not possible to correct for this in the earlier set of experiments where only optical clearing was followed. In this series there was, however, less clearing in plasma from all the cases of essential hyperlipemia than the normals. In the second set of experiments both optical clearing and release of FFA were measured. In the system for FFA determination, such an influence of a different initial fat content of the plasmas should have been excluded.

Klein, Lever and Fekete (1959) made no efforts to equalize the concentrations of fat in their system with hyperlipemia and normal plasmas. However, as the diminished optical clearing was accompanied by diminished production of glycerol and a less rapid increase in the mobility of lipoproteins in their hyperlipemics, their evidence for actual inhibitors in hyperlipemia seems rather strong.

In 5 of 6 cases of essential hyperlipemia the optical clearing was lower and in 4 of 6 the release of FFA was lower than in normals. It is felt that the problem of eventual plasma inhibitors in essential hyperlipemia has not been satisfactorily studied for reasons quoted above. It can also be objected against the present studies that it was not certain that the triglyceride content in the plasma of the hyperlipemics was tending to rise when the experiments were performed. This is probably relevant because of the rapid changes that for unaccountable reasons occur in essential hyperlipemia. A future study should take these changes in the hyperlipemic state into account. However, the existence of inhibitory factors in plasma is at least probable in several of the cases investigated.

Summary

In this series of patients with essential hyperlipemia, lipoprotein lipase activity as measured 5, 10 and 15 minutes after a small dose of heparin was as good as and in a few cases better than in normals and in cases of nonhyperlipemic atherosclerotics, as followed by a decrease in optical density *in vitro*. After a large dose of heparin (100 mg) in cases of essential hyperlipemia, the lipoprotein lipase activity *in vitro* might appear as low or absent. Most probably this was due to consumption of the available fatty acid acceptor capacity by virtue of the high amount of substrate present in essential hyperlipemia. This phenomenon was illustrated by using a milieu with inhibitor freed plasma.

As measured *in vitro* at the same concentration of post-heparin clearing factor the optical clearing of a fat emulsion was less in essential hyperlipemia than in normals. Release of FFA was usually but not consistently less in hyperlipemic plasma.

Chylomicra from cases with essential hyperlipemia proved to be more resistant to clearing than chylomicra obtained after a fat meal from normals. In one patient the chylomicra in the fasting state in the morning were more resistant than the chylomicra obtained after a fat meal in the same patient. The cholesterol content in the chylomicra in essential hyperlipemia seemed consistently high.

Of interest is the comparison of chylomicra from the case E A. in the fasting and the post alimentary state In this case we have interpreted the chylomicra obtained in the post-alimentary state as being a mixture of "fasting and "post alimentary chylomicra. The chylomicra obtained in the post alimentary state cleared much better than the "fasting' ones The difference in chylomicra between essential hyperlipemia and normals raised the question whether the chemical composition of the chylomicra was identical. This question has not been closely pursued. However we performed a study of the cholesterol content of the chylomicra in essential hyperlipemia.

Albrink, Man and Peters (1955) found when centrifuging lactescent serum from cases of essential hyperlipemia, nephrosis and diabetic acidosis, that when the neutral fat in serum was high, the greatest part of cholesterol was in the creamy layer (sometimes 70—80 % or more) Cholesterol and total fat in the creamy layer of plasma centrifuged at 25 000 *g* for 60 minutes was determined by substrating the values in the subnatant from the values in uncentrifuged serum The cholesterol content was expressed as per cent of total fat.

The results are seen in table IV The cholesterol content of the chylomicra calculated in this way seems high in the hyperlipemic subjects as compared with the normals given by Jobst and Schettler (1956) In two of these patients (S Ö and H. K.) the cholesterol content of the chylomicra was determined before and after a large injection of heparin 100 mg intravenously This led to a marked increase in the percentage cholesterol content of their chylomicron phase.

Discussion

Plasma from patients with essential hyperlipemia showed when assayed *in vitro* in comparison with normal plasma, at least the same and in some instances a greater optical clearing after a small dose of heparin This contrasts with the findings obtained in the study of the P family by Havel and Gordon (1960) However the clinical features of the P family are also very dissimilar to those ordinarily found in essential hyperlipemia.

The resistance of chylomicra from hyperlipemic patients to clearing as assayed *in vitro* is in agreement with the findings of Carlson and Wadström (1956) in one case. Albrink, Man and Peters (1955) found chylomicra with a high cholesterol content in essential hyperlipemia, diabetic acidosis and nephrosis. In a study by French, Robinson and Harris (1953) cholesterol-fed rabbits had a high cholesterol content in their chylomicra and these showed resistance to clearing with post heparin plasma. The high cholesterol content in the chylomicron fraction in our series of essential hyperlipemia patients may have significance in this respect

The sharp further increase in the cholesterol content of the chylomicra 1 hour after a large dose of heparin may reasonably be interpreted according to either of two alternatives Either there was a selective removal of triglycerides from the chylomicra or there was a selective removal of those chylomicra which contained less cholesterol

However while this feature of relative resistance to clearing of the chylomicra in essential hyperlipemia is a striking one it seems difficult to conceive of this as a primary pathogenetic feature.

The sets of experiments designed to study plasma inhibitors of clearing are

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On the Commonness, Causes and Detrimental Effects of the Use of Analgesics by Factory Workers and Hospital Patients

By

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The use of analgesics and tranquillisers has increased considerably since world war II. In Switzerland, the increase in the use of analgesics has been 3-4-fold (Schweingruber 1955) According to an investigation made in Denmark, 29 4 per cent of hospital patients use phenacetin daily and, in addition, 22 3 per cent occasionally (Larsen and Moller 1959)

The most important aspect of the problem of analgesics is their role as a causative agent of renal damage. Renal lesions are typical especially of phenacetin, and were first demonstrated by Spühler and Zollinger in 1953. This observation has since been confirmed by several workers in Switzerland and Scandinavia (Zollinger 1945 Moeschlin 1957 Nordenfelt and Ringertz 1961) A very detailed study has also been made of the pathoanatomical picture of chronic interstitial nephritis and papillitis necroticans (Hultengren 1961 Nordenfelt and Ringertz 1961) Toxic lesions in the kidney can also be caused by other analgesics, e. g acetylsalicylic acid, phenylbutazone and acetalinid (Clausen and Harvald 1961)

The practical risk of renal damage involved in the abuse of analgesics was indicated from a series in which the continuous use of large daily quantities of phenacetin resulted in renal damage in 47 per cent (Schweingruber 1955)

Renal function was impaired in 53.2 per cent of a series of hospital patients who used phenacetin daily in 10.9 per cent of the patients who used it occasionally and in 8 6 per cent of those who did not use it at all (Larsen and Moller 1959)

To obtain an idea of the anamnestic significance of the use of phenacetin, the present authors conducted a comparative study of the use of analgesics in a large factory among hospital patients and especially among patients with renal disease under hospital treatment.

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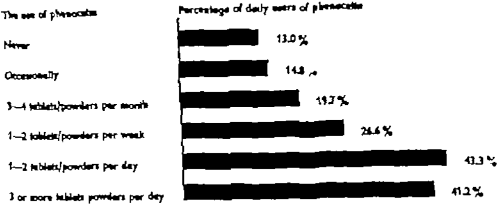


Fig. 1. The use of analgesics and the frequency of renal disease.

Reason for starting to take phenacetin and when started

The reason was analysed in the factory series. The study covered all persons taking phenacetin regularly. Headache seems to have been by far the most important reason for beginning the habit (table II). Pain did not in itself lead to the abuse of phenacetin for it was not used in the hospital series any more frequently by patients with rheumatoid arthritis than by other patients.

It might be thought, of course, that the exceptionally liberal use of analgesics by patients with renal disease is due to the symptoms of renal disease, back pain and above all the general symptoms of uremia. To test this, the authors questioned the renal patients about the time when they started to take analgesics in relation to the onset of urinary tract symptoms. The use of analgesics had begun distinctly earlier than the first urinary tract symptoms in two-thirds of the cases. On the other hand, it must be remembered that sometimes renal diseases are accompanied by very few symptoms in the initial phase and have an insidious onset.

Frequency of renal diseases in users of analgesics (industrial population)

The authors examined 100 patients who had indulged in analgesics for a minimum of 3 years. Eighty-seven were men, 13 women. The mean hemoglobin for the men was 13.4 g %, against 13.6 g % for the control group of the same population. For the women the means were 12.2 % for phenacetin users and 12.5 % in the control group.

The urinary sediment finding was indicative of urinary tract infection in 2 women and 10 men, i.e. 12 % of these 100 patients. One, moreover, had permanent proteinuria. The blood pressure was elevated in 8 cases.

The hospital patients

Eight hundred patients admitted successively to the medical department were distributed into groups by the amount of phenacetin used. It was clear that the use of analgesics grew more common with the increase in the incidence of renal diseases (fig. 1). Eighty-five % of the patients who had consumed over 5 kg of phenacetin suffered from renal disease only 13 % of the patients who did not use phenacetin were similary affected.

Use of analgesics by patients with renal disease

As was seen in table I, patients with renal diseases used analgesics in a considerably greater degree than other patients. The authors also endeavoured to find out how this use was distributed between different diagnostic groups (table III) and established that analgesics were used liberally by pyelonephritis patients in particular. The group uremia non definita, which is taken to represent probable interstitial nephritis group, also used phenacetin in considerable quantities. No renal biopsies were performed.

Table I The frequency of the use of analgetics in the different groups

Use of analgenics	Industrial workers (total 2 004) %		Control group (total 612) %		Renal diseases group (total 350) %	
	Women	Men	Women	Men	Women	Men
Never	12.6	16.5	18.7	29.3	13.3	23.1
Occasionally	37 4	48.5	45.5	39.5	31 4	33.7
3—4 tablets/powders per month	24.8	19 7	10.3	8.1	8.2	10.5
1—2 tablets/powders per week	12.6	7 6	9.0	8.2	9 8	6.4
1—2 tablets/powders per day	9.6	5.4	11.5	6.2	23.1	17.8
3 tablets/powders per day	3.0	2.3	5.0	8.1	14.2	8.5

Material and methods

The first group consisted of the workers of a large industrial establishment. Replies were received from 2 004 persons, i. e. 72 1 ⁄ Of these, the 100 greatest phenacetin users were tested as regards hemoglobin, sedimentation rate and serum creatinine. The second group consisted of 800 successive medical patients admitted to the hospital. A special blank form was prepared to ascertain their use of analgenics. From this series were adopted as a special control group the 612 patients whose history or status revealed no symptoms of renal disease The third group consisted of 350 successive hospital admissions for renal disease. As the sex distribution differs considerably in these groups, women and men were treated separately The mean age was 44 years for the factory workers, 49 years for the non renal diseases group and 46 years for the renal diseases group

Table II Reasons for commencing analgenics

	%
Headache	45.5
Back pain	15.2
Joint pain	14.2
Prevention of colds	7.3
As tranquillising medicine	5.9
Not feeling well	5.5
For the feeling of well-being induced by the drugs	3.2
Menstruation trouble	1.8
Some other reason	1.2
Cannot say	0.2

Frequency of use of phenacetin

The use of phenacetin was very common in all the groups (table I) but especially so among the patients with renal disease. The percentage of women among factory workers taking phenacetin daily was 12.6, in the non renal diseases group 16.5, and in the renal diseases group 37.3 For men, the corresponding percentages were 7 7 14.3 and 26.3. The differences are statistically significant.

Effect of age and sex

In all the groups, women used phenacetin distinctly more than men. This varied with age The maximum use for women was at the age of 40—50 when the daily consumption was double that of the younger age groups. For men the peak came 10 years later The physical and psychic symptoms associated with the menopause obviously tended to increase the use of analgenics. A definite decrease occurred after the 60th year

Effect of occupation

There could conceivably be an interdependence between the use of analgenics and social conditions. The authors analysed this problem in a series of hospital patients. The incidence of daily users of analgenics was equally high in all social groups. Whether th women were at work or not made no difference. Nor v ere differences established between urban and rural dwellers.

cent. It appeared that the abuse must continue for a fairly long time before the renal lesions mature and that by no means all users have renal symptoms.

Of the patients with renal diseases, only those with chronic pyelonephritis and non-definite nephropathy took phenacetin daily in unusually large amounts, 55 and 85.9 per cent, respectively. No differences from the control group were established in the other renal diseases.

The use of phenacetin is surprisingly common and the users are not aware of the dangers. Many persons have withdrawal symptoms on discontinuing phenacetin.

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Table III The frequency of the use of analgetics in the different diagnostic groups

Diagnosis	No. of patients	Daily takers of phenacetin %
Pyelonephritis acuta	79	7 6
Pyelonephritis chronica	131	56.0
Nephritis ac	15	—
Nephritis chron.	21	10.0
Nephrolithiasis	3	—
Tbc renis	6	—
Nephropathia diabetica	35	11 4
Nephrosclerosis	11	18.0
Nephropathia non definita	38	84.4
Hypertrophia prostata	4	—
Anomalies	5	—
Nephropathia gravidarum	3	—
Non-renal diseases group	612	16.8

Discussion

The abuse of phenacetin can already be regarded as a veritable common disease. Ten per cent of the employees at a large industrial establishment, 20 per cent of a series of hospital patients, 35 per cent of the patients with renal disease and 55 per cent of chronic pyelonephritis patients used analgesics daily

A distinct difference is demonstrable in the incidence of renal diseases between the industrial population and the hospital population. The picture obtained from the hospital series is considerably worse than that of non hospital persons who abuse phenacetin the latter gave an almost normal frequency of renal disease. It seems obvious from this that only a minority of the abusers are finally affected with renal diseases Man can obviously tolerate about 1 000 g of phenacetin daily for 5—10 years without damage, if he is lucky On the other hand taking the renal disease group of the hospital the phenacetin abusers seem to predominate among renal patients. Earlier experience has shown that abuse of phenacetin must continue for 7—10 years before it causes renal damage.

A number of the patients used analgesics for the ensuing sensation of well-being and definite withdrawal symptoms were encountered in some of the patients who gave up the habit.

Summary

The series consisted of 2,004 factory workers, 612 hospital patients with no symptoms of renal disease and 350 hospital patients with renal diseases. They were all questioned concerning the use of phenacetin the reasons for so doing and the time at which they began to use them Of the factory workers, the 100 biggest takers of phenacetin were studied separately for the presence of renal diseases.

The percentage of female factory workers taking analgesics daily was 13 4 of men 7 8 per cent. In the non renal diseases group of the hospital series the comparable figures were 16 5 and 14 3 per cent, and in the renal diseases group 37 3 and 26 3 per cent Women indulged most in analgesics at the age of 40—50 Occupation did not affect the issue.

The main reason for starting the habit was headache (45 5 per cent of the series)

Of the daily takers of analgesics at the factory 13 per cent had renal disease, of the hospital patients 42.5 per-

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Postural Circulatory Changes at Rest and during Exercise in five Patients with Congenital Absence of Valves in the Deep Veins of the Legs

By

S. Bevegård and A. Lodin

A change of body posture from recumbency to erect position causes a shift of blood from the thorax to the lower part of the body (12, 17). This results in a decrease of the heart volume (9) and the stroke volume (12) probably as a consequence of lower effective filling pressure. Transition from rest to exercise in the sitting position causes an increase of the heart volume (9) and the stroke volume (3, 4) to levels slightly below those obtained in supine position, indicating a redistribution of blood in a central direction. The venous pump of the leg muscles, the sympathetic tone adjusting the volume of the venous system and the abdominothoracic pump (16) seem to be factors of importance for this redistribution. Patients with funnel chest have been found to have an abnormally low stroke volume during exercise in sitting position. The underlying mechanism for this is unknown, but it has been suggested that a reduced efficiency of the abdomino-thoracic pump might be a possible explanation (2). With onset of leg exercise in the erect position, contraction of the leg muscles can be expected to accelerate the venous flow and reduce the volume of blood contained in the leg veins, the latter effect being indicated by a reduced venous pressure at the ankle (15, 10, 11). The efficiency of the "muscular pump" is dependent on intact venous valves. In an attempt to determine the role of the venous valves for the maintenance of the stroke volume, five patients with congenital absence of venous valves have been examined with heart catheterization at rest and during exercise in supine and sitting position.

Material and methods

Four women and one man with congenital absence of venous valves have been studied. The clinical findings in these cases have been reported in detail by Lodin (14) and phlebographic examinations by Lindvall and Lodin (13). According to the phlebographic examinations, three cases (no. 1—3) had no demon-

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From the Laboratory of Clinical Physiology Thoracic Clinics and the Department of Dermatology Karolinska Sjukhuset, Stockholm, Sweden

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Table I. Some anthropometric data in 5 cases with congenital absence of valves in the deep leg veins

Case no.	Sex	Age years	Height cm	Weight kg	B. S. A. m	Heart volume ml	Total hemoglobin g	Hemoglobin conc g/100 ml	Blood volume l
1	F	24	164	54.0	1.59	590	470	10.3	4.6
2	F	34	168	62.2	1.71	700	665	12.4	5.4
3	F	42	163	55.0	1.54	575	575	12.2	4.7
4	F	35	171	54.0	1.65	580	535	10.6	5.1
5	M	20	184	70.0	1.94	835	630	12.6	5.0

Not in "steady state". 4th I. S. = 4th intercostal space. 0 = avalvular. af = abnormally few valves.

strable venous valves in the upper or lower extremities. Two cases (no. 4, 5) had a few (1—3) valves per venous trunk in the lower leg. Except for recurrent leg ulcers all patients were healthy. There were no signs of cardiac disease. None of the patients had any varicosities. Some anthropometric data of the material are presented in table I.

Methods, methodological errors and procedure for examinations have earlier been reported in detail (3). Right heart catheterization was performed without premedication. Between the investigation in sitting and supine posture the patient rested for at least 30 min. After this time restitution of pulse rate and oxygen uptake has usually occurred according to earlier experience (3, 4). Cardiac output was determined according to the direct Fick method.

Results

The total *hemoglobin* (THb) per kg body weight was within normal limits (6) in all cases except for no. 5 where it was slightly low. The *hemoglobin concentration* was slightly low in case no. 1, 4 and 5. The *blood volume* per kg body weight in all cases was within normal limits (7). The *heart volume* in the prone position in relation to the THb in all cases was within the range of two standard errors of estimate from the normal regression line (6). The *pulse rate* at rest after 8 minutes standing (orthostatic test) did not show abnormally high values in any of the cases (6). The *pulse rate during exercise* in the three cases with complete absence of venous valves (no. 1—3) was considerably higher in sitting than in supine position on identical work loads. On the highest work load the pulse rate was 11—24 beats higher in sitting than in supine position. This difference resulted in lower values for the rate of work at a pulse rate of 150 and 170 beats per min in the sitting than in the supine position (table I). In the two cases, who had a few venous valves in the legs, no

Work intensity kpm/min. at pulse rate 170 beats/min. (above) and 150 beats/min. (below)		Pulse rate (supine) beats/min.	Pulse rate (standing) beats/min.	Venous pressure at the ankle mm Hg			Hydrostatic pressure 4th I. S. manometer mm Hg	Incidence of valves in the leg veins
Sitting	Supine			Supine	Standing	Walking		
370 240	320 400	90	104	8	86	86	79	0
600 430	730 590	76	92	7	87	87	77	0
660 500	— 600	84	108	7	88	84	81	0
880 680	780 600	68	72	10	89	65	88	af
1,060 840	1,070 850	77	88	—	—	—	—	af

such difference was found. In cases 1 and 2, the work intensity in sitting position at a pulse rate of 170 beats/min. was definitely lower than expected from other measures of the circulatory dimensions, such as heart volume and total hemoglobin (6).

Venous pressure at the ankle was measured in four cases at rest in supine position, at rest, standing, and during walking (table I). In the three cases with complete absence of venous valves it did not decrease during walking. In case no. 4 who had a few venous valves in the legs it decreased significantly.

Data obtained during heart catheterization

The *pulse rate at rest* was considerably higher than found prior to heart catheterization only in case no. 1. At the second determination at rest, supine, only case no. 2 showed a markedly higher value. At rest, sitting on the bicycle, the pulse rate increased slightly or was essentially unchanged.

The *pulse rate during work* on the highest load was in all cases higher in sitting than in supine position. The difference, however, was pronounced only in case no. 1, 2 and 5. Prior to catheterization, case no. 1, 2 and 3 had shown this reaction, but not case no. 5.

The *oxygen uptake at rest* in supine position was 14–38 per cent higher than the predicted basal value (3). It was within the same range during the second determination.

The *oxygen uptake during work* was approximately the same in sitting and supine position on identical work loads (fig. 1). The mechanical efficiency varied between 22 and 25 per cent on the maximum work load employed and was on an average equal in sitting and supine position.

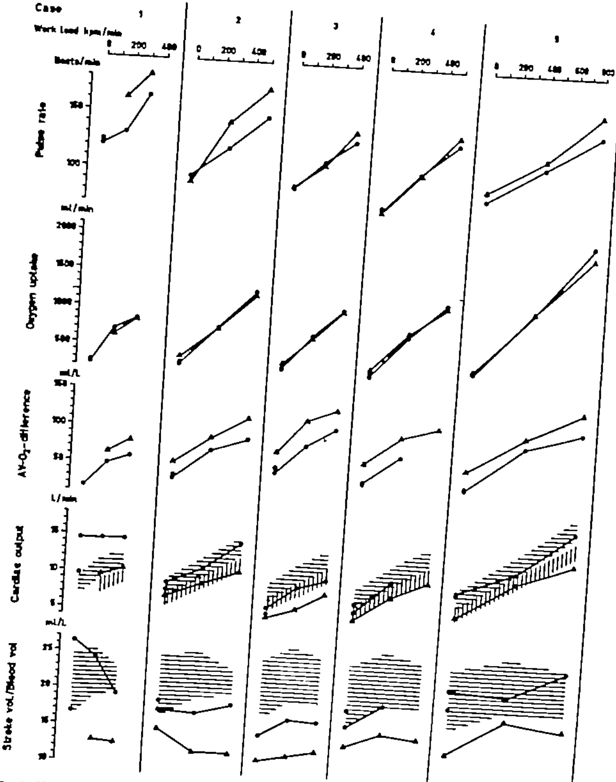


Fig 1 Hemodynamic changes t rest and during exercise in five patients with congenital bsence o venous val es in the legs. Filled circles represent alues in supin and triangles alues in sitting pou tion. The hatched areas represent the normal variations within ± one standard error of est mat horizontal lines refer to supine, nd ertical lines to sitting position (3, 8)

The *oxygen saturation of the arterial blood* varied within normal limits at rest as well as during exercise (3 8)

The *oxygen saturation of mixed venous blood* at rest and during exercise in all cases was lower in the sitting than in the supine position. Case no. 1 who was anxious and had a high pulse rate at rest and during exercise, showed a saturation higher than normal in relation to pulse rate (3 8)

The *arterio-venous oxygen difference*, as in normal cases (3) was higher in each case in sitting than in supine position. In case no. 1 it was abnormally low in relation to pulse rate at rest and during exercise, both in supine and sitting position. Case no. 5 showed a somewhat high arterio-venous oxygen difference especially in sitting position, when compared to a group of normal subjects (3 8) The other cases showed fairly normal variations at rest as well as during exercise.

The *cardiac output* at rest and during exercise in all cases was lower in sitting than in supine position. In cases no. 1 2 and 5 the difference on the highest work load was larger than the average for normal subjects (3) Case no. 1 showed a large cardiac output in relation to oxygen uptake during the first determination at rest, but tended to return to normal during exercise. During the second determination at rest in supine position, however the cardiac output was fairly normal in relation to oxygen uptake. In case no 5 the cardiac output was somewhat low in relation to oxygen uptake at rest and during exercise both in supine and sitting position

The *stroke volume* in supine position in relation to blood volume was large in case no. 1 during the first determination at rest, but within normal limits during work and the second determination at rest (fig 1) In supine position it was somewhat small in relation to blood volume in case no. 2 and 3 but was well within normal limits in case no. 4 and 5 At rest, on changing the posture from supine to sitting the stroke volume decreased by 17—33 per cent which is somewhat less than in normal subjects (3) On transition from rest to exercise, while sitting, it increased only slightly and in case no. 2 even decreased. The mean values for the stroke volume during work were 22—42 per cent lower in sitting than in supine position. The difference was most pronounced in the cases with complete absence of venous valves. In supine position the mean values for the stroke volume during work in relation to heart volume and in relation to total hemoglobin in all cases were within the range of two standard errors of estimate from the normal regression lines (3 8)

The *intracardiac and intravascular pressures* (table II) were within normal limits in all cases (3) The pressures in the sitting and supine position are not directly comparable, as the reference levels are not exactly the same in the two body positions.

Discussion

An investigation involving a procedure such as heart catheterization may through the psychological reaction of the patient change the circulatory dynamics. Judging from the values for pulse rate and oxygen consumption, this occurred to substantial extent in case no. 1 This patient was very anxious and had a considerably higher pulse rate during heart catheterization than before. At rest in the sitting position no determinations were obtained in this patient because she felt faint. Especially during the first determination at rest in supine position the cardiac out

Table II. Data obtained in connection with right heart catheterization in 5 cases with congenital abscence of

Case no.	Position	Work load kpm/min.	Pulse rate beats/min.	Oxygen uptake ml/min.	Mechanical efficiency %	O_2-capacity ml/100 ml	O_2-sat. %		AV-O_2 diff ml/l
							Br A.	PA	
1	R	rest	120	253		14.3	97	86	17
	R	150	131	694	14	14 7	98	67	48
	R	300	165	841	22	14.5	98	60	53
	R	rest	124	255		14.2	99	81	27
	S	150	161	607	17	15.3	96	56	64
	S	300	182	830	22	15.6	98	48	80
2	R	rest	78	272		17 7	100	81	35
	S	rest	89	353		18.4	98	71	52
	S	250	143	756	22	19 1	98	53	87
	S	500	173	1,207	24	19.7	96	39	115
	R	rest	93	260		17.3	99	82	30
	R	250	120	757	22	17 7	99	60	70
	R	500	149	1,258	23	18.2	99	52	87
3	R	rest	82	257		17.5	99	70	52
	S	rest	89	323		18.0	98	60	72
	S	200	108	671	21	18.8	98	38	116
	S	400	139	1 048	23	19.3	96	29	131
	R	rest	88	255		16.3	98	72	44
	R	200	112	706	20	17.0	97	50	82
	R	400	131	1 055	23	17 7	97	38	107
4	R	rest	74	238		15.0	98	76	35
	S	rest	70	295		16.1	97	59	63
	S	250	104	803	20	16.9	99	41	100
	S	500	139	1 164	25	17.9	97	35	114
	R	rest	71	215		14.8	98	74	37
	R	250	105	757	22	15.3	96	49	74
	R	500	132	1,210	24	16.6	97	—	—
5	R	rest	92	316		18.4	100	81	36
	S	rest	92	343		19.2	96	66	59
	S	400	122	1 145	22	20.0	96	44	106
	S	800	163	1,893	24	20.5	98	31	141
	R	rest	85	314		18 4	100	82	34
	R	400	115	1 145	22	19 0	100	52	94
	R	800	145	2,051	22	19.3	97	39	114

S = sitting R = recumbent. Br A. = brachial artery RV = right engide. PA = pulmonary artery

put was abnormally large in relation to oxygen consumption. During exercise the deviation from normal tended to decrease. As no methodological or technical errors could be discovered and as there were no signs of any left to right shunt we believe that the hyperkinetic circulation in this case may have been evoked by the procedure. With regard to the influence of body position during work, however the performance of this patient was essentially the same as that of the others The

valves in the deep leg veins

Card. output l/min.	Stroke volume ml	Pressures, mm Hg								
		RV		PA			PCV	Br. A.		
		S	De	S	D	M		S	D	M
14.5	121	27	3	27	5	13		108	69	90
14.5	111	31	6	30	6	18		134	75	102
14.5	88	36	1	36	3	14		130	62	82
9.6	77	21	2	19	3	9		115	66	89
8.5	59	30	−1	29	6	15		133	80	95
10.4	57	50	4	33	6	17		130	71	87
7.7	99	23	4	17	7	12	8	117	73	94
6.8	77	25	5	24	10	15		125	77	100
8.7	61	46	2	33	10	18		130	81	103
10.3	61	56	1	38	9	20		146	77	100
8.7	92	20	1	12	3	9	6	114	68	90
10.8	90	32	3	24	11	19		125	89	96
14.4	97	43	4	31	9	18		138	71	101
4.9	60	25	6	23	10	17	9	127	74	99
4.5	50	21	3	20	10	13		134	86	112
5.8	53	34	0	25	10	14		144	73	106
8.0	57	44	−2	29	11	19		163	73	109
5.8	66	24	5	22	10	16	10	132	73	97
8.7	77	33	6	31	13	21	14	149	73	103
9.9	76	45	6	36	13	24		170	73	119
6.9	93	25	7	23	8	13		99	58	73
4.7	67	20	1	18	7	11		112	61	84
8.1	77	37	1	23	9	14		121	60	91
10.3	74	50	0	—	—	21		132	62	92
5.6	81	23	6	20	9	14	8	104	54	72
10.2	97	44	10	34	13	22	14	123	56	83
—	—	51	4	45	12	24		146	62	95
8.8	96	29	5	22	7	14	8	121	75	85
5.8	64	22	3	20	9	11		119	80	96
10.8	89	43	—	28	8	15		147	80	101
13.3	83	55	4	33	12	20		189	80	120
9.2	109	28	6	24	10	15	11	124	73	97
12.2	106	38	4	24	10	17	11	151	74	103
10.6	124	52	2	52	9	17		186	77	117

PCV = pulmonary capillary venous. S = systolic, De = end-diastolic, D = diastolic, M = mean.

other four cases did not show any remarkable deviations from normal in the supine position.

At rest in the erect position, the superficial and deep veins of the legs are distended by a pressure slightly higher than the pressure of a hydrostatic column extending up to the level of the heart (15, 10). This implies that at rest the blood is flowing continuously in the venous system and all valves are open. After onset of leg exercise in the erect position

Table II Data obtained in connection with right heart catheterization in 5 cases with congenital absence of

Case no.	Position	Work load kpm/min.	Pulse rate beats/min.	Oxygen uptake ml/min.	Mechanical efficiency %	O_2-capacity ml/100 ml	O_2-sat. %		AV-O_2 diff ml/l
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S = sitting R = recumbent. Br A. = brachial artery RV = right ventricle PA = pulmonary artery

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noted in the two patients, who had occasional valves in the veins of the legs.

3 The stroke volume during exercise was considerably smaller in sitting than in supine position. The difference, which appeared to be abnormally large in the three cases with complete absence of venous valves, explained the higher pulse rate during exercise in sitting than in supine position on identical work loads.

4 The small stroke volumes during exercise in an upright position can be explained by an impaired ability to redistribute blood from the legs to the central circulation on transition from rest to exercise, due to a reduced efficiency of the venous pump.

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the pressure in the superficial and in the deep veins of the calf normally decreases considerably (10). Thus the veins become less distended with onset of exercise, which may indicate that a shift of blood from the legs to the central circulation has occurred, resulting in a subdivision of venous pressure into shorter hydrostatic columns, separated by closed venous valves.

Absence of venous valves or valvular incompetence can be expected to diminish such a shift of blood, and thus the distribution of the blood volume during exercise in the erect position would remain approximately the same as at rest.

In the present material of patients with congenital absence of venous valves, the most striking hemodynamic finding was a stroke volume that during exercise was considerably lower in sitting than in supine position. The difference, expressed as a percentage, was on an average significantly larger ($p < 0.01$) than in normal subjects (3). It was somewhat more pronounced in the three cases with complete absence of venous valves, where the stroke volume during exercise was an average of 35 % lower in sitting than in supine position, compared to an average of 12 % for normal subjects. In case no. 2 the stroke volume even decreased on transition from rest to exercise in the sitting position. The low stroke volumes during work in sitting position are probably explained by impaired ventricular filling due to inadequate redistribution of blood from the legs to the central circulation on transition from rest to exercise. This seems to be supported by the fact that the venous pressure at the ankle did not decrease during leg exercise in the avalvular cases.

The effect that can be developed during work at a given pulse rate is predominantly determined by the size of the stroke volume (4). Consequently a small stroke volume in the sitting position must decrease the maximal working capacity and to some extent also the rate of work at a given pulse frequency as is illustrated in case no. 1 and 2. However a small stroke volume can be compensated for, within some limits, by an increase in the peripheral oxygen utilization as in case no. 3.

Arenander (1) found in a material of patients with varicose veins a higher pulse rate during work in sitting than in supine position on identical work loads. It seems reasonable to explain this observation with the assumption of a similar mechanism as in the cases studied in this report.

The present investigation seems to indicate that with complete absence of venous valves in the legs the stroke volume during exercise in an upright position is smaller than ordinary in respect to the circulatory dimensions of the individual. Thus the observation seems to demonstrate the significance of the venous valves for the circulatory adaptation to different demands.

Summary

1. In five patients with congenital absence of valves in the veins of the leg the circulation has been studied with the aid of right heart catheterization at rest and during exercise in sitting and supine position. The results have been compared with data obtained from normal subjects investigated during identical conditions.

2. Three patients with complete absence of venous valves showed during exercise a substantially higher pulse rate in sitting than in supine position on the same work loads. This difference was not

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From the Department of Physiological Chemistry[1] (Head B. Borgström, M. D.) and the Department of Medicine[2] (Head H. Malmros, M. D.) University of Lund, Sweden

The Content of Total and Acid-soluble Glycogen in the White Blood Cells and its Relation to the Staining with the Periodic Acid-Schiff Reagents

By

A. Dahlqvist[1] G. Gahrton and Å. Nordén

In blood smears from diabetic patients the lymphocytes (Mähr 1959) and the neutrophil granulocytes (Gartner & Nordén 1961) both show increased staining by the periodic acid-Schiff (PAS) technique, when compared with smears from non-diabetic subjects. Since the PAS positive material is removed on pre incubation with amylase, it has been supposed to consist of glycogen.

Determinations of the glycogen content of white blood cells by chemical methods in diabetic patients have, however shown either no increase (Valentine, Follette & Lawrence 1953 Esmann 1961) or subnormal values (Esmann 1961).

In order to evaluate the correlation between the PAS staining and the chemically determined glycogen content, we have examined the white blood cells employing both types of technique in parallel studies.

Supported by grants from Edith Carlssons fond, E. & T. Sandins minnesfond, Svenska Diabetesforbundet and from the Swedish Medical Research Council.

Submitted for publication December 11 1961

Clinical material

The clinical material consisted of sixteen non-diabetic subjects and nineteen patients with diabetes mellitus. The absence of diabetes was established by blood sugar determinations only. Glucose tolerance tests were unfortunately not performed. In the present study this was not considered so important since the main interest was in the relation between the PAS staining and the content of glycogen. The question of the PAS staining in cases with an abnormal glucose response without overt diabetes is at present under extended study. Eighteen determinations were performed in the non-diabetic group and twenty-seven in the diabetic one. In addition determinations of acid-soluble glycogen were made in ten "non-diabetics" and thirteen diabetics.

Group A. Sixteen "non-diabetic" subjects: five were apparently healthy persons (nurses and medical students), six were patients with congenital heart disease, one had mitral stenosis, three were undergoing treatment for gastric ulcer (one of these patients was later found to be suffering from prostatic carcinoma with skeletal metastases). Finally one patient was regarded as psychoneurotic.

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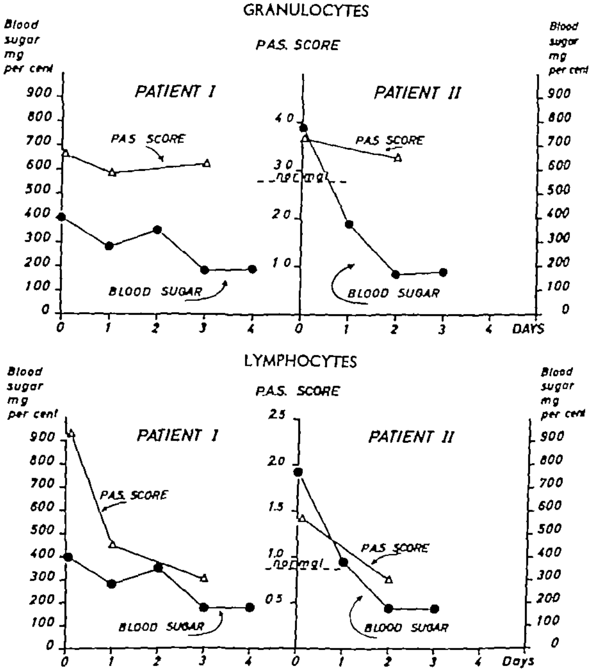


Fig 1 Decrease in the PAS score of the white blood cells in two diabetic patients with ketosis during adequate treatment The decrease was most marked in the lymphocytes (lower curve) The results of chemical determination of glycogen in these cases are seen in table IV

Group B (Satisfactorily controlled diabetic patients — mean daily blood sugar 150 mg per cent or lower) This group consisted of eight patients. None had ketonuria. Two patients showed occasional glycosuria. Six were being treated with insulin, one with chlorpropamide and one by diet only

Group C (Poorly controlled diabetic patients — mean daily blood sugar higher than 150 mg per cent) This group consisted of twelve patients. Nine of them had glycosuria and (or) ketonuria. At the time of study eleven of them were being treated with insulin, one was on diet only

Two patients with ketosis were studied separately (see fig. 1 and table IV). Patient No. I (fig. 1) is included in the group of poorly controlled diabetics. In patient Nr II (fig. 1) the chemical determinations of glycogen yielded values above the standard curve (see table IV), thereby rendering accurate estimations impossible. This patient has not been included in the series used for the comparison between the PAS staining and the glycogen content.

One patient was studied as member both of group B and of group C.

In the diabetic patients blood sugar determinations were made 2—4 times a day and the mean blood sugar was calculated for 5—7 days before the investigation. In some unsatisfactorily controlled cases the investigation was performed immediately on arrival at hospital. In such cases the classification was based on the initial blood sugar value.

In the non-diabetic" subjects blood sugar determinations were made twice day for 3 days fasting at 7[00] a. m., and at 3[00] p. m. three hours postprandially

All blood sugar determinations were performed according to Hagedorn et al. (1935)

Methods

Sampling

Finger tip puncture for peripheral blood smears (for PAS staining) and venous puncture for the separation of white blood cells (for chemical glycogen determination) were performed simultaneously in the morning with the patient in the fasting state.

PAS staining method

The smears were air-dried, *fixed in absolute* methyl alcohol for 10 minutes, rinsed in distilled water and stained with the PAS technique, as described by Gartner & Nordén (1961)

In an attempt to quantitate the PAS staining the scoring system of Astaldi et al. (1952) was used. Granulocyte staining was graded according to the intensity of diffuse staining exhibited by the cytoplasm. The lymphocytes were graded by recording the density of PAS positive granules in each cell, in categories 0 to 4 One hundred cells of each kind were examined, and the score calculated according to the formula

$$\frac{\times 0 + b \times 1 + c \times 2 + d \times 3 + \times 4}{100} = \text{score}$$

In this formula a, b, c, d and e represent the number of cells belonging to the various categories (0—1—2—3—4) out of one hundred cells.

Evaluation of the PAS stained smears was usually carried out within a *few days of* preparation, and always by the same person who had no knowledge of the nature of the investigation.

Separation of white blood cells

About 10 ml of blood obtained by venous puncture was transferred to a test tube containing 5 ml of isotonic fibrinogen solution (1 per cent fibrinogen, 1 per cent sodium chloride, 0.1 per cent disodium-ethylenediaminetetraacetate (EDTA) buffered with sodium citrate and sodium acetate to pH 6.5) (Kabi AB, Stockholm, Sweden) and 25 mg of heparin (Vitrum A. S., Copenhagen, Denmark) The tube was placed at an angle of 60° from the horizontal plane at room temperature (20° C) for 75—90 minutes for the red blood cells to settle. Centrifugation was not used as this was found to diminish the total yield. The supernatant containing white blood cells and platelets was removed and used for chemical glycogen determination. The number of mononuclear (lymphocytic) and polymorphonuclear (granulocytic) cells in the cell suspension was counted in duplicate or triplicate in Buerker counting chamber The cell suspension was mixed carefully before removing samples, in order to avoid settling of the cells. The number of white blood cells per mm³ of cell suspension varied between *4 000 and 30,000 depending* on the patient white blood cell count. The relation between mononuclear and polymorphonuclear cells in the white blood cell suspension obtained by this method was pproximately the same as found in the blood smears from the patient. The cell suspension also contained platelets, which so far have been disregarded. The importance of the platelets can not, therefore, be evaluated t present. The small amount of red blood *cells present in the* suspension could be neglected as it was later found that the red cells contain no detectable amounts of glycogen.

Variation of the sedimentation time between 45 and 115 minutes did not alter the amount of glycogen found per 10^{10} white blood cells. Fibrinogen and heparin in the amounts used gave negligible reaction with the anthrone reagent.

Chemical determination of total glycogen in white blood cells

One ml of the white blood cell suspension was centrifuged at 800 × g for 10 minutes, and the sedimented cells were suspended in 3.0 ml of 30 per cent (w/v) sodium hydroxide. The tubes were covered with inverted small beakers, heated in a boiling water bath for 45 minutes and cooled with tap water. After adding 5.1 ml of 95 per cent (v/v) ethyl alcohol the contents of each tube were mixed with a glass rod, and left for at least 30 minutes. The glycogen was sedimented by centrifugation at 1,500 × g for 10 minutes at room temperature, resuspended in 6.0 ml of 60 per cent (v/v) ethyl alcohol and again sedimented by centrifugation. The washing was repeated once. Finally the glycogen was dried by heating the tubes in an oven at 100° C.

The glycogen in each tube was suspended in 2.0 ml of distilled water and then quantitatively determined with the anthrone reagent as described by Scott & Melvin (1953) but with the sole modification that heating was performed for 7.5 minutes at 100° C instead of 16 minutes at 90° C. In order to avoid loss of material the entire procedure was performed in the same test tube. In actual control experiments the transfer of resuspended glycogen precipitate to another test tube was followed by a 20 to 35 per cent loss due to the adherence of the glycogen to the glass walls.

The amount of glycogen present in each sample was estimated from a standard curve based on the extinction values obtained with quantities of glucose from 50 to 150 µg, assuming that 0.9 µg of glycogen would yield the same reading as 1.0 µg of glucose. From the number of white blood cells present in the cell suspension, the amount of glycogen was expressed in mg per 10^{10} of mixed white blood cells, or in mg per 10^{10} granulocytes or 10^{10} lymphocytes. The figures were obtained by dividing the amount of glycogen found by the total number of white cells or the total number of granulocytes or lymphocytes. Thus in the latter instances all the glycogen was assumed to be present in the granulocytes or in the lymphocytes. In the correlation analysis of the variations in the PAS score with the chemically determined glycogen content, further assumptions about the relative distribution of the glycogen in the granulocytes or in the lymphocytes had to be made, using values obtained by the formula described below (page 37).

All determinations of glycogen in white blood cells were made in triplicate, with satisfactory agreement between the different samples.

As a check of the method the whole procedure was applied to weighted samples of commercially obtained glycogen (from Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.). The recovery was 90 per cent.

Chemical determination of the acid-soluble glycogen fraction in white blood cells

The white blood cells in a 2.0 ml cell suspension were sedimented by centrifugation at 800 × g for 10 minutes. The supernatant was discarded. The cells were resuspended in 0.9 ml of distilled water and the tubes were immersed in crushed ice for 15 minutes. One ml of ice cold 20 per cent (w/v) trichloroacetic acid was added and the tubes were kept in the ice bath for another 60 minutes with occasional stirring with a glass rod. The tubes were then centrifuged at 800 × g for 10 minutes at room temperature. One ml of the clear supernatant containing the acid-soluble glycogen was transferred to another test tube. The tube was immersed in ice and 2.0 ml of 95 per cent (v/v) ethyl alcohol was added with thorough mixing. After 30 minutes the glycogen was sedimented by centrifugation at 1,500 × g for 10 minutes. The glycogen was resuspended in 3.0 ml of 30 per cent (w/v) sodium hydroxide in water. It was heated in a boiling water bath, precipitated and washed with ethyl alcohol, dried and determined quantitatively by the anthrone method as previously described in the procedure for the determination of total glycogen. Triplicate determinations were made on each sample.

Table I. PAS score for granulocytes and lymphocytes in "non-diabetic" and diabetic subjects. The granulocyte score was significantly higher in group C than in group A ($p = 0.01$) and group B ($0.02 < p < 0.05$). The lymphocyte score seems to be higher in the two diabetic groups (B and C) than in the "non-diabetic" group (A), but the difference was not statistically significant ($0.05 < p$). The two patients with ketosis had high scores in both kinds of white blood cells

Group	Number of determinations	PAS score	
		Granulocytes	Lymphocytes
"Non-diabetic" (A)	18	2.78 ± 0.06	0.87 ± 0.09
Diabetics			
Satisfactorily controlled (B)	16	2.78 ± 0.10	1.06 ± 0.15
Poorly controlled (C)	17	3.04 ± 0.05	1.04 ± 0.12
In ketosis	2	3.31 3.68	2.35 1.41

Standard error of the mean.

In control experiments using commercially obtainable glycogen recovery of 91 per cent was found.

The amount of acid-soluble glycogen present was calculated as a percentage of the total glycogen.

Results

PAS staining

The PAS scores for the granulocytes showed increased values in poorly controlled diabetics (patients of group C) when compared with the scores found in the "non-diabetic" group (A) and the satisfactorily controlled diabetics (B) as seen from table I.

The PAS scores of the lymphocytes were somewhat higher in the two groups of patients with diabetes (B and C) than in the "non-diabetic" group (A). The difference was, however, not statistically significant in the lymphocytes.

In two cases with diabetic ketosis scores were found to be high both in the granulocytes and in the lymphocytes (fig. 1). Following treatment with insulin a decrease was noted in both groups of cells within the next few days. The decrease was less in the granulocytes than in the lymphocytes.

Chemical determination of total glycogen in white blood cell mixtures

In assessing the results of the chemical determinations of glycogen, consideration had to be given to possible differences in glycogen content between granulocytes and lymphocytes, since the determinations were performed on mixtures of both types of cells.

Assuming each granulocyte to contain the same quantity of glycogen as each lymphocyte, then the three groups of patients studied showed no difference (table II). If, however, the glycogen found in the cell mixture were supposed to originate entirely from the granulocytes as Valentine et al. (1953) and Esmann (1961) have assumed, then significantly lower values were found in patients with poorly controlled diabetes. In patients with diabetes under satisfactory control, values lower than in the normal group were recorded. The difference was, however, not statistically significant. Table III gives the results and also, for comparison, the values found by Valentine et al. (1953) and Esmann (1961). The range in mg per 10^{10} cells is in our studies higher than found by Valentine et al.

Variation of the sedimentation time between 45 and 115 minutes did not alter the amount of glycogen found per 10^{10} white blood cells. Fibrinogen and heparin in the amounts used gave negligible reaction with the anthrone reagent.

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The PAS scores for the granulocytes showed increased values in poorly controlled diabetics (patients of group C) when compared with the scores found in the "non-diabetic" group (A) and the satisfactorily controlled diabetics (B) as seen from table I.

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Table II Chemically determined (total) glycogen content of white blood cells, calculated per 10^{10} mixed cells or per 10^{10} granulocytes or 10^{10} lymphocytes When the amount of glycogen was calculated for the mixed cells, no difference was found between the three groups (A B and C) studied When it was calculated per 10^{10} granulocytes group C had a significantly lower value than group A ($p = 0.01$) while group B had an intermediate value which was not significantly different from any of the two other groups. When the glycogen was calculated per 10^{10} lymphocytes, group C had a significantly higher value than group A ($p < 0.01$) and group B ($p < 0.01$) while the latter two groups did not differ significantly from each other

Group	Number of determinations	Total glycogen mg per 10^{10} cells		
		Total white blood cells	Granulocytes	Lymphocytes
"Non-diabetic" (A)	18	91 ± 4.7	162 ± 8.9	241 ± 21.5
Diabetics				
Satisfactorily controlled (B)	10	83 ± 7.4	140 ± 14.6	217 ± 22.1
Poorly controlled (C)	17	88 ± 5.1	125 ± 10.4	354 ± 31.2

Standard error of the mean.

Table III Comparison of the glycogen content of white blood cells calculated in mg per 10^{10} granulocytes in our series with values found by Valentine et al (1953) and by Esmann (1961)

	Total glycogen mg per 10^{10} granulocytes		
	Valentine et al. (1953)	Esmann (1961)	Present series
"Non-diabetic subjects	75.1 ± 17.4	148 ± 9	162 ± 8.9
Diabetic subjects			
Satisfactorily controlled	–	156 ± 12	140 ± 14.6
Poorly controlled	78.5 ± 26.9	111 ± 10	125 ± 10.4

Standard deviation. Standard error of the mean.

(1953) but of the same magnitude as found by Esmann (1961) The reason for the difference is as yet not clear

Leikin (1961) has recently claimed that the lymphocytes contain the major part of the glycogen found in leukocyte mixtures. This assumption when applied to our data, gives significantly higher values in the group with poorly controlled diabetes than in the group with controlled diabetes and in the non-diabetic group (table II) Such an interpretation would suggest a parallel increase of the glycogen content and of the intensity of the PAS reaction in the lymphocytes. It would, however leave unexplained the increased PAS score observed in the granulocytes.

In the two patients with ketosis illustrated in fig 1 the chemically determined glycogen was high in the lymphocytes assuming the granulocytes did not contain any glycogen (table IV) There was a tendency for this to decrease during treatment with insulin Calculations based on the assumption of an equal distribution of the glycogen between granulocytes and lymphocytes, or of its presence in only the granulocytes, gave low values in patient No I

Table IV Chemically determined (total) glycogen content of white blood cells in two patients with diabetic [illegible]. The PAS scores in these patients are seen in fig 1. The glycogen content when calculated per 10^{10} lymphocytes was high in both cases, with a tendency to decrease during treatment. The glycogen content calculated for mixed white blood cells or for granulocytes only was low in patient I. No such conclusion may be drawn from the data for patient II

Case	Days of treatment	Total glycogen mg per 10^{10} cells		
		Total white blood cells	Granulocytes	Lymphocytes
Patient I	0	64	73	500
	1	54	66	306
	3	80	97	455
Patient II	0	> 80	> 89	> 770
	2	86	124	400

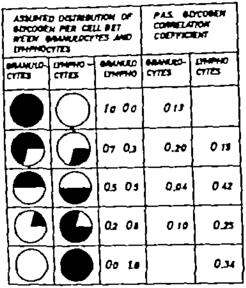


Fig 2 Correlation between PAS score for the granulocytes and the lymphocytes, respectively and the amount of glycogen present in isolated white blood cells, calculated with different assumptions as to the relative mean glycogen content of a granulocyte and a lymphocyte. No significant correlation was found between the PAS score of the granulocytes and the chemically determined glycogen content, but the lymphocytes showed a significant positive correlation between PAS score and glycogen content if a lymphocyte was assumed to contain at least as much glycogen as a neutrophil granulocyte.

Correlation between the PAS stain and the chemically determined glycogen

Correlation analysis were performed using the pooled data from the three groups of patients representing a total of 45 determinations. The product moment coefficient of correlation was calculated (Yule & Kendall 1950). Analysis of the correlation between the chemically determined amount of glycogen and the intensity of the PAS reaction were performed on the basis of various possibilities for the relative distribution of glycogen within the granulocytes and the lymphocytes. The following formula was employed

$$X = \frac{}{G + \frac{1}{q} L}$$

in which X represents the glycogen content of a single granulocyte

— mg glycogen per mm³ of the leukocyte suspension

G = number of granulocytes per mm³ in the leukocyte suspension

L = number of lymphocytes per mm³ in the leukocyte suspension

q = quotient assumed for $\frac{\text{glycogen per single granulocyte}}{\text{glycogen per single lymphocyte}}$

The glycogen content of a single lymphocyte then amounts to $\frac{x}{q}$

No significant correlation was obtained between the scores for the PAS reaction in the granulocytes and the amount of chemically determined glycogen in any one of the various possibilities for the glycogen quotient tested (fig 2)

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Group	Number of determinations	Total glycogen mg per 10^{10} cells		
		Total white blood cells	Granulocytes	Lymphocytes
"Non-diabetic" (A)	18	94 ± 4 7	162 ± 8.9	241 ± 21.5
Diabetics				
Satisfactorily controlled (B)	10	83 ± 7 4	140 ± 14 6	217 ± 22.1
Poorly controlled (C)	17	88 ± 5.1	125 ± 10.4	354 ± 31.2

Standard error of the mean.

Table III Comparison of the glycogen content of white blood cells calculated in mg per 10^{10} granulocytes in our series with values found by Valentine et al. (1953) and by Esmann (1961)

	Total glycogen mg per 10^{1} granulocytes		
	Valentine et al. (1953)	Esmann (1961)	Present series
"Non-diabetic" subjects	75.1 ± 17 4	148 ± 9	162 ± 8.9
Diabetic subjects			
Satisfactorily controlled	–	156 ± 12	140 ± 14.6
Poorly controlled	78.5 ± ?6.9	111 ± 10	125 ± 10.4

Standard deviation. Standard error of the mean.

(1953) but of the same magnitude as found by Esmann (1961) The reason for the difference is as yet not clear

Leskin (1961) has recently claimed that the lymphocytes contain the major part of the glycogen found in leukocyte mixtures. This assumption when applied to our data gives significantly higher values in the group with poorly controlled diabetes than in the group with controlled diabetes and in the non-diabetic group (table II) Such an interpretation would suggest a parallel increase of the glycogen content and of the intensity of the PAS reaction in the lymphocytes. It would, however leave unexplained the increased PAS score observed in the granulocytes.

In the two patients with ketosis illustrated in fig 1 the chemically determined glycogen was high in the lymphocytes, assuming the granulocytes did not contain any glycogen (table IV) There was a tendency for this to decrease during treatment with insulin Calculations based on the assumption of an equal distribution of the glycogen between granulocytes and lymphocytes, or of its presence in only the granulocytes, gave low values in patient No. 1

cell mixtures. The question arises whether the granulocytes contain some PAS-positive substance which is not glycogen. Against such an assumption is the observation that the stainable material is, as far as we have been able to observe, completely removed by treatment with pure amylase. Analysis of cell suspensions containing only one type of cell seems necessary.

The possibility that the platelets may contribute to the glycogen content of the cell mixture also has to be considered. Platelets are strongly PAS-positive. Wagner (1946) reported that the platelets contained no measurable amounts of glycogen. Esmann (1961) using a technique which differs somewhat from ours, made determinations on samples containing no platelets. Our results for the chemically determined glycogen content per cent are of the same order of magnitude as found by Esmann (1961) which suggests that the platelets in our estimations did not contribute any appreciable amount of glycogen.

The glycogen present in animal tissues has been divided in two different fractions by Bloom et al. (1951) one acid-soluble which can be extracted by cold trichloroacetic acid and the other acid-insoluble. Kugler and Wilkinson (1960) and Wilkinson & Kugler (1961) found that in liver sections only the acid-soluble fraction would react with the PAS reagents. In the present studies the acid-soluble fraction was found to represent about 60 per cent of the total amount of glycogen in all three groups of persons — non-diabetic controlled diabetes and uncontrolled diabetes. The results do not support the assumption that the PAS reaction is better correlated with the content of acid-soluble glycogen than with total glycogen.

Summary

1. The periodic acid-Schiff (PAS) reaction of the leukocytes in peripheral blood smears from diabetic and "non-diabetic" subjects has been studied by a semi-quantitative scoring system. The PAS score for the granulocytes was found to be significantly higher in patients with poorly controlled diabetes than in "non-diabetic" persons and patients with diabetes under control. The PAS scoring for the lymphocytes also showed an increase in diabetic subjects but the difference when compared to normal persons was not statistically significant.

2. Chemical determinations of the glycogen content of white blood cells obtained from the same groups of patients showed no change in diabetic subjects. When the results were related to the granulocytes only the group of poorly controlled diabetes showed a lower content of glycogen than the "non-diabetics" or controlled diabetics. If the results were related only to the lymphocytes then uncontrolled diabetes entailed an increase of the glycogen content.

3. The correlation between the intensity of the PAS reaction and the glycogen content necessitated consideration of various possible distributions of glycogen between the granulocytes and the lymphocytes. Various assumptions were tested mathematically. In no instance could any correlation be demonstrated between the glycogen content of the granulocytes and the staining intensity.

A statistically significant correlation was demonstrable in the lymphocytes on the assumption that the glycogen content per lymphocytic cell was equal to or larger than the content per granulocytic cell.

The lymphocytes on the other hand, showed a significantly positive correlation between the scoring results and the glycogen content, assuming either an equal distribution ($q = 1$) or its presence mainly in the lymphocytes ($q < 1$).

Chemical determination of acid-soluble glycogen

The acid-soluble glycogen fraction amounted to between 41 and 85 per cent of the total glycogen present in the cell mixtures. There was no significant difference in the relative amount of acid soluble glycogen in the three groups of subjects studied. The mean values expressed as a percentage of the total amount of glycogen were: in the "non-diabetic" group (A) 63 ± [1]4.8, in the satisfactorily controlled diabetic group (B) 60 ± 2.6, in the poorly controlled diabetic group (C) 66 ± 2.2.

Discussion

The results of the PAS staining in the present studies are in accordance with previous findings in which significantly increased staining was observed in material from diabetic patients in the lymphocytes by Mähr (1960) and in the granulocytes by Gärtner and Nordén (1961).

Evaluation of the results of the chemical determinations of glycogen in white cell mixtures requires calculation of the glycogen content of the granulocytes and lymphocytes separately. Most previous authors have regarded the granulocytes as the major site of white blood cell glycogen (Valentine et al. 1953, Esmann 1961). This assumption seems to have been based on microscopic observation of stained cells. Recently, however, Leikin (1961) published results of chemical determinations of the content of glycogen in lymphocyte-rich white cell suspensions suggesting that the lymphocytes probably accounted for the main part of the glycogen in granulocyte-lymphocyte mixtures.

Our results in the three groups of subjects viz. "non-diabetic" persons and patients with diabetes (controlled and uncontrolled) depend on the assumptions made for the distribution of the glycogen between the granulocytes and the lymphocytes in the cell mixture. If the glycogen is assumed to be localized only in the granulocytes, then poorly controlled diabetes is followed by a decrease, but if the lymphocytes contain all the glycogen, then cases of uncontrolled diabetes (group C) show higher values than "non-diabetics" or cases of diabetes under proper control. In neither case could any significant difference be established between "non-diabetics" or diabetics under satisfactory control.

Analysis of the relation between the amount of glycogen and the PAS reaction showed no significant correlation in the granulocytes whatever distribution was assumed for the glycogen between granulocytes and lymphocytes.

A statistically significant positive correlation between the glycogen content and the PAS reaction was, however, found in the lymphocytes if the lymphocytes as compared with the granulocytes were assumed to contain as much or more glycogen per cell.

These results have been held to suggest that the granulocytes, in spite of their strong PAS reaction, may not represent the main source of the glycogen in white

[1] Standard error of the mean.

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From the Second University Clinic of Internal Medicine Kommunehospitalet,
Aarhus, Denmark

Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method

II. The Values in Undiluted and Diluted Serum from Diabetic Patients Determined before and after the Ingestion of Glucose

By

J. Lyngsøe

In a previous study (Lyngsøe 1962) the results were reported of an examination of the insulin-like activity of serum (SILA) in a group of normal subjects. The main conclusions were that when the serum is diluted, SILA increases, and that after the ingestion of glucose, SILA is higher than in the fasting state in both undiluted and diluted serum.

A number of laboratories studying SILA levels in diabetic patients have found values that varied considerably (Bornstein et al. 1951 Vallance-Owen et al. 1955 Willebrands et al. 1956, Seltzer & Smith 1959 Rizzo et al. 1961 Pfeiffer et al. 1959 1961 Yalow & Berson 1960 Steinke et al. 1961) Using an in vivo method (the ADHA rat method) and an in vitro method (the rat diaphragm method) a number of investigators (Bornstein et al. 1951 Vallance-Owen et al. 1955 Seltzer & Smith 1959 Rizzo et al. 1961) have been able to demonstrate a difference in SILA values in patients suffering from different "types" of diabetes. However in other studies using the rat diaphragm method (Willebrands et al. 1956, Randle 1957) and studies using the rat epididymal fat method and the immunological method (Steinke et al. 1961 Yalow & Berson 1960) no such differences could be demonstrated.

In the studies mentioned very divergent results for the SILA level were obtained in patients suffering from diabetes mellitus, possibly because undiluted serum was used in some of the investigations and diluted serum in others. In view of this, I have in the present study using the rat epididymal fat method, examined both undiluted and diluted serum from a large number of diabetic subjects, representing a chance selection among the patients admitted to a medical department.

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4 Determinations of the acid-soluble fraction of the glycogen showed a constant proportion of about 60 per cent in all groups

5 The results seem to indicate that the PAS-reactive material in the granulocytes is not necessarily identical with glycogen while in the lymphocytes the PAS-positive material may consist mainly of glycogen

The necessity of investigating suspensions containing only one type of cell rather than mixtures of granulocytes and lymphocytes is apparent

Acknowledgement

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J. LYNGSOE

In a previous study (Lyngsoe 1962) the results were reported of an examination of the insulin-like activity of serum (SILA) in a group of normal subjects. The main conclusions were that when the serum is diluted, SILA increases, and that after the ingestion of glucose, SILA is higher than in the fasting state in both undiluted and diluted serum.

A number of laboratories studying SILA levels in diabetic patients have found values that varied considerably (Bornstein et al. 1951 Vallance-Owen et al. 1955 Willebrands et al. 1956 Seltzer & Smith 1959 Rizzo et al. 1961 Pfeiffer et al. 1959 1961 Yalow & Berson 1960 Steinke et al. 1961) Using an in vivo method (the ADHA rat method) and an in vitro method (the rat diaphragm method) a number of investigators (Bornstein et al. 1951 Vallance-Owen et al. 1955, Seltzer & Smith 1959 Rizzo et al 1961) have been able to demonstrate a difference in SILA values in patients suffering from different "types" of diabetes. However in other studies using the rat diaphragm method (Willebrands et al. 1956 Randle 1957) and studies using the rat epididymal fat method and the immunological method (Steinke et al. 1961 Yalow & Berson 1960) no such differences could be demonstrated.

In the studies mentioned very divergent results for the SILA level were obtained in patients suffering from diabetes mellitus, possibly because undiluted serum was used in some of the investigations and diluted serum in others. In view of this, I have in the present study using the rat epididymal fat method, examined both undiluted and diluted serum from a large number of diabetic subjects, representing a chance selection among the patients admitted to a medical department.

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Table I Clinical data on patients with diabetes mellitus

Patient no.	Sex	Age	Age at onset	Height cm	Weight kg	Ideal weight kg	Weight %	Insulin I. u./24 hours
58	M	58	58	167	62	68.8	<100	—
64	F	51	44	144	44	49.6	<100	—
65	F	68	53	159	61	60.5	101	40
70	F	66	63	166	73	65.8	111	12
77	M	65	63	184	99	82.6	120	—
89	M	62	49	163	59	66.8	<100	52
90	F	78	70	172	83	70.3	118	20
91	F	55	55	160	64	61.3	104	— (later Tolbutamide)
92	F	68	67	162	70	62.8	111	— (later Tolbutamide)
96	F	67	58	162	60	62.8	<100	— (Carbutamide)
98	M	38	38	163	56	66.8	<100	20
99	M	22	22	181	68	80.4	<100	40
102	M	64	59	169	86	71.3	121	32
104	M	61	51	170	78	72.1	108	—
105	F	72	72	155	71	57.5	124	—
108	F	43	42	168	66	67.3	<100	16
109	M	38	38	168	70	70.6	<100	44
112	M	59	49	171	73	72.8	100	24
115	M	15	15	181	54	—	—	60

Duration of insulin treatment	Last dose of insulin before blood sampling i. u.	Blood sugar on day of examination 0700, 1300, 1700 mg/100 ml	Urine after examination	24-hours urine on day of examination	Total CO mEq/l
—	—	191 212 164	1.8 % −G−L	17 g −G−L	—
—	—	184	—	4 g −G−L	—
7 years	28 insulin lente 12 insulin semilente 120 hours before examination	128-140-105	—	4 g −G−L	—
13 day	24 protamin insulin 48 hours before examination	152-185-286	1.3 % −G−L	16 g −G−L	—
—	—	119-150-97	<0.4 % −G−L	− 8 −G−L	—
13 years	44 protamin insulin 24 hours before examination	91 385-272	0.9 % −G−L	12 g −G−L	—
2 years	20 protamin insulin 120 hours before examination	193-292-183	1.2 % −G−L	10 g −G−L	—
—	—	272 316-282	2.0 % −G−L	10 g −G−L	—
—	—	173-230-183	1.9 %	10 g −G−L	—
—	Carbutamide 1 g 144 hours before examination	—	—	7 g −G−L	—
6 days	20 protamin insulin 24 hours before examination	170	1.2 % −G−L	—	—
—	—	—	1.8 % −G+L	—	—
3 years	32 protamin insulin 24 hours before examination	—	0.7 % −G−L	18 g −G−L	—
—	—	95-99-107	0.4 % −G−L	6 g −G−L	—
—	—	101 142 78	0.9 % −G−L	− 8 −G−L	—
1 year	16 protamin insulin 144 hours before examination	132 154-169	1.6 % −G−L	7 g −G−L	—
3 days	24 regular insulin 24 hours before examination	193-328-204	2.9 %	38 g −G−L	—
10 years	24 protamin insulin 144 hours before examination	162 240-181	0.9 % −G−L	13 g −G−L	—
10 days	32 regular insulin 24 hours before examination	181 234-173	2.3 % −G−L	16 g −G−L	—

Table I (cont)

Patient no.	Sex	Age	Age at onset	Height cm	Weight kg	Ideal weight kg	Weight	Insulin i. u./ 24 hours
120	M	61	61	173	124	74.5	169	—
133	F	57	49	165	80	65.1	123	56
134	F	78	70	159	7	60.5	124	28
135	M	23	3	168.5	55	71	<100	28
136	F	79	73	152	63	55.2	114	—
149	M	73	62	174	67.5	75.1	<100	—
153	F	7?	63	153	66	55.9	118	20 (later Chlorpropamide)
152	F	57	57	154	59	56.6	104	32
151	M	13	13	164	59	—	—	56
161	F	53	53	15.	63	55.2	114	—
162	M	39	34	18'	101	81 1	1.5	24
181	F	40	38	162	53	62.8	100	20
182	M	26	25	189	80	86.4	<100	44
185	F	71	70	155	71	57 5	139	12 (later Tolbutamide)
188	M	50	45	180	74	79.6	<100	12
190	F	67	47	166	66	65.5	101	20
191	F	65	63	155	55	57.5	<100	—
192	F	36	17	159	58	60.5	<100	40
193	M	13	15	164	54	—	—	36

Duration of insulin treatment	Last dose of insulin before blood sampling I. u.	Blood sugar on day of examination 0700 1300, 1700 mg/100 ml	Urine after examination	24-hours urine on day of examination	Total CO_2 mEq/l
—	—	204–268–175	0.9 % −G−L	−G−L	—
8 years	32 regular insulin 24 hours before examination	163–258–240	0.9 % −G−L	16 g −G−L	—
6 days	28 protamin insulin 48 hours before examination	224–350–246	1.4 % −G−L	36 g −G−L	—
—	—	248–373–268	5.6 % −G−L	50 g −G−L	—
—	—	268–332 280	1.8 % −G−L	24 g −G−L	—
—	—	150–240–164	1.7 % −G−L	7 g −G−L	—
13 days	20 protamin insulin 72 hours before examination	294–238–189	—	5.7 g −G−L	—
—	—	236–494–270	+ 8 −G+L	30 g −G+L	24
1 year	24 regular insulin 24 hours before examination	109–109–36	0.9 % −G−L	7 g −G−L	—
—	—	152 242 177	0.9 % −G−L	10 g −G−L	—
5 years	24 regular insulin 24 hours before examination	164–197 130	1.8 % −G−L	16 g −G−L	
1 year	20 insulin lente 48 before examination	228–310–186	2.1 % −G−L	22 g −G−L	—
—	—	270–384–163	2.7 % −G−L	34 g −G−L	—
½ year	24 protamin insulin 24 hours before examination	163–150–163	0.8 % −G−L	9 g −G−L	—
5 years	12 protamin insulin 48 hours before examination	182 203–122	2.8 % −G−L	−G−L	—
20 years	20 protamin insulin 48 hours before examination	238–510–240	1.7 %	34 g −G−L	—
—	—	236–264–252	1.5 „ −G−L	14 g −G−L	—
19 years	24 regular insulin 17 hours before examination	384–431 311	3.5 „ −G+L	18 g −G+L	20
—	—	174–420–283	3.2 % −G−L	65 g −G−L	26

Table I (cont.)

Patient no.	Sex	Age	Age at onset	Height cm	Weight kg	Ideal weight kg	Weight %	Insulin I. u./24 hours
120	M	61	61	173	124	4.3	169	—
133	F	57	49	165	80	63 1	123	56
134	F	78	70	159	75	60.5	124	28
135	M	93	23	168.5	55	71	<100	28
136	F	79	73	152	63	55.2	114	—
149	M	73	62	174	67.5	75.1	<100	—
153	F	72	63	153	66	55.9	118	20 (later Chlorpropamide)
152	F	57	57	154	59	56.6	104	32
151	M	13	13	164	59	—	—	56
161	F	33	33	15	63	55.2	114	—
162	M	39	34	182	101	81 1	125	4
181	F	40	38	162	53	62.8	100	20
182	M	26	25	189	80	86.4	100	44
185	F	71	70	155	71	57 5	139	12 (later Tolbutamide)
188	M	50	45	180	74	79.6	<100	12
190	F	67	47	166	66	65.5	101	20
191	F	65	63	155	55	57.5	<100	—
192	F	36	17	159	58	60.5	<100	40
193	M	15	15	164	54	—	—	36

Duration of insulin treatment	Last dose of insulin before blood sampling i. u.	Blood sugar on day of examination 0700, 1300, 1700 mg/100 ml	Urine after examination	24-hours urine on day of examination	Total CO_2 mEq/l
13 years	32 regular insulin 24 hours before examination	411 315–233	–	40 g −G−L	–
6 years	16 regular insulin 24 hours before examination	323–420–301	3.8 % −G−L	100 g	–
–	–	117 135–119	1.0 % −G−L	14 g −G−L	–
–	–	177–252 258	5.7 % −G−L	30 g −G−L	–
–	–	208–250–204	1.6 % −G−L	19 g −G−L	–
–	–	127–108–109	0.9 % −G−L	4 g −G−L	–
–	–	100–94–110	0.6 % −G−L	− 5 +G−L	–
–	–	162 77–110	<0.4 % −G−L	− 8 −G−L	–
–	–	190–244–268	1.7 % −G−L	24 g −G−L	–
–	–	241–452 317	3.1 % −G−L	2.3 % −G−L	–
–	–	163–180–147	1.5 % −G−L	10 g −G−L	–
2 years	40 protamin insulin + 20 regular insulin 24 hours before examination	393–206	1.6 % −G−L	–	–
6 years	32 protamin insulin 20 hours before examination	259–273–194	1.2 % −G−L	58 g −G−L	–
7 days	20 protamin insulin 24 hours before examination	173–117 53	2.1 % −G−L	31 g −G−L	–
–	–	255–434–460	3.5 % +G+L	150 g +G+L	25
–	–	411 267–197	3.5 % −G+L	28 g −G+L	24
–	–	212–408–514	1.8 % −G+L	70 g −G+L	31

Table I (cont.)

Patient no.	Sex	Age	Age at onset	Height cm	Weight kg	Ideal weight kg	Weight %	Insulin i. u./24 hours
194	F	52	39	165	77	65.1	118	64
195	M	55	30	172	61	73.6	<100	32
197	M	55	55	181	84	80.4	104	—
202	M	62	53	166	83	69.1	120	— (later Tolbutamide)
203	F	55	54	156	74	58.3	127	—
200	M	56	51	161	84	65.3	129	—
204	F	68	68	167	80	66.6	120	—
208	F	63	62	152	81	55.2	147	—
215	F	64	53	162	77	62.8	123	— (later Chlorpropamide)
218	F	46	43	160	85	61.3	139	32
230	F	51	45	163	135	63.6	212	—
246	F	24	22	161	71	62.1	114	88
247	M	36	30	187	80	84.9	<100	84
248	M	30	30	182	64	81.1	<100	20
249	F	16	16	169	52	68.1	<100	36
250	M	13	13	155	42	—	—	32
251	M	23	23	192	63	88.5	<100	24

—G = no acetoacetic acid.

—L = no acetone.

Duration of insulin treatment	Last dose of insulin before blood sampling i. u.	Blood sugar on day of examination 0700, 1300, 1700 mg/100 ml	Urine after examination	24-hours urine on day of examination	Total CO mEq/l
13 years	32 regular insulin 24 hours before examination	411 315–233	—	40 g −G−L	—
6 years	16 regular insulin 24 hours before examination	323–428–301	3.8 % −G−L	100 g	—
—	—	117–155–119	1.0 % −G−L	14 g −G−L	—
—	—	177–232 258	3.7 % −G−L	30 g −G−L	—
—	—	208–258–204	1.6 % −G−L	10 g −G−L	—
—	—	127–108–109	0.9 % −G−L	4 g −G−L	—
—	—	100–94–110	0.6 % −G−L	− S +G−L	—
—	—	162 77 110	<0.4 % −G−L	− S −G−L	—
—	—	198–244–268	1.7 % −G−L	24 g −G−L	—
—	—	241–432 317	3.1 % −G−L	2.3 % −G−L	—
—	—	163–188–147	1.5 % −G−L	10 g −G−L	—
2 years	40 protamin insulin + 20 regular insulin 24 hours before examination	393–206	1.6 % −G−L	—	—
6 years	32 protamin insulin 20 hours before examination	259–273–194	1.2 % −G−L	58 g −G−L	—
7 days	20 protamin insulin 24 hours before examination	175–117–55	2.1 % −G−L	31 g −G−L	—
—	—	255–434–460	3.5 % +G+L	150 g +G+L	25
—	—	411 287–197	3.5 % −G+L	28 g −G+L	24
—	—	212–408–314	1.8 % −G+L	76 g −G+L	31

Table II SILA values from patients with diabetes mellitus

Assay no.	Patient no.	Serum glucose mg	SILA, undiluted serum µU/ml		SILA, diluted serum µU/ml		Lambda	No. of rats
			Fasting	After glucose	Fasting	After glucose		
Onset age < 25								
208	99	185—277	23	15	160	105	0.23	3
234	115	46—399	31	61	55	150	0.28	4
255	135	329—550	24	36	0	0	0.50	4
258	151	142—315	20	29	230	310	0.32	3
462	192	518—724	20	54	180	180	0.36	3
294	193	201—436	70	210	110	165	0.39	4
551	46	416—620	105	69	115	145	0.27	4
Onset age 25—40								
227	98	190—491	32	61	780	100	0.18	4
233	109	61—398	0	22	0	55	0.43	3
268	162	224—409	18	18	0	0	0.22	4
283	181	264—458	93	150	120	235	0.19	4
284	182	284—567	120	265	145	575	0.14	4
93	194	400—542	32	83	110	1 5	0.26	4
296	195	316—376	23	52	100	930	0.35	4
532	247	282—456	145	130	625	175	0.23	4
554	248	188—356	118	110	490	460	0.11	4
Mean value ± standard dev Juvenile diabetics			54.7 ± 47	85 6 ± 72	169.9 ± 172	233 1 ± 245	—	—
Onset age > 40, Non-obese								
162	58	162—299	11	27	0	0	0.22	4
166	64	156—321	232	380	100	500	0.19	4
171	65	133— 73	73	160	183	240	0.34	4
176	70	164— 45	64	66	130	65	0.26	4
198	91	206—290	26	70	60	180	0.20	4
202	92	180—298	61	120	230	180	0.23	4
203	96	107—190	68	200	135	235	0.26	4
213	104	102—216	135	192	425	330	0.19	3
217	108	129—298	36	100	130	200	0.35	3
221	112	161—228	110	140	220	193	0.27	4
223	89	108—250	43	60	50	90	0.27	4
242	136	321—365	45	212	65	153	0.46	4
256	149	175—332	58	64	60	85	0.23	4
260	152	248—354	65	120	103	195	0.23	4
267	161	174—328	14	22	0	0	0.56	4
290	188	148—324	163	183	425	760	0.25	4
291	191	246—376	220	245	540	280	0.22	4
292	190	305—472	340	40	255	260	0.31	4
298	197	155—320	41	38	250	125	0.56	3
Mean value ± standard dev Old non-obese diabetics			93.1 ± 87	138.9 ± 93	178.2 ± 150	214.5 ± 178	—	—
Mean value Non-obese diabetics			76.7 ± 73	114.6 ± 87	174.3 ± 158	223 1 ± 207	—	—

Table II. (cont.)

…ary …	Pa-tient no.	Serum glucose mg %	SILA, undiluted serum µU/ml		SILA, diluted serum µU/ml		Lambda	No. of rats
			Fasting	After glucose	Fasting	After glucose		
…set age > 40. Obese								
185	77	125—239	27	79	0	130	0.26	3
201	90	162—319	170	180	125	410	0.19	3
211	102	140—336	150	1 100	330	1,200	0.19	4
214	105	192—177	200	272	460	780	0.26	3
228	120	221—291	295	235	215	410	0.21	4
238	153	242—384	270	330	650	1,250	0.24	4
240	134	246—304	90	142	295	225	0.18	4
259	153	188—332	46	140	110	605	0.28	4
287	185	173—424	240	410	400	535	0.28	4
301	200	104—244	145	245	725	775	0.34	3
303	202	191—327	152	305	515	625	0.28	3
304	203	202—407	160	290	340	1,250	0.34	4
305	204	192—250	120	240	550	500	0.47	4
407	208	134—213	360	540	500	975	0.33	4
432	213	198—330	48	59	0	0	0.23	4
448	218	248—445	110	225	825	480	0.33	4
477	230	209—350	285	320	325	560	0.12	4
Mean value ± standard dev. Obese diabetics			167.5 ± 95	300.7 ± 238	386.2 ± 245	618.2 ± 379	—	—

Material and methods

The material consists of both newly diagnosed and long-standing cases of diabetes. None of the patients examined had malignant disease or jaundice and all were afebrile at the time of examination. None of the patients had diabetic coma. Patients exhibiting ke tonuria had total blood CO_2 of more than 19 mEq/l

The patients were hospitalized, except for pt. no. 99 who was examined as out-patient after rest in bed for one hour. The patients under insulin treatment received their last dose of insulin lente semilente or NPH-insulin not later than 36 hours before examination, and their last dose of regular insulin not later than 24 hours before examination.

This scheme was not maintained in some patients, as Nos. 89, 98, 102, 185, 246, 247 and 248 received NPH-insulin 24 hours before examination.

Blood samples for SILA determinations were drawn from an arm vein, raising the venous pressure slightly by tourniquet. A fasting blood sample was taken between 7 and 8 a. m. after fasting and rest in bed for 8—12 hours. The patients were then given an aqueous solution of glucose to drink (1 g glucose/kg body weight). The patients continued resting in bed, and 1 / hours after administration of the glucose solution a further sample of venous blood was drawn for SILA determination. In patients with ketonuria, the total CO was also deter mined in the same sample of blood as was used for SILA determination.

SILA is determined by a modification of the rat epididymal fat method[1] (Lyngsøe 1961). SILA values found to be below 10 µU/ml are reported as 0 µU/ml. The serum for determining SILA is prepared from venous blood, as described in a previous study (Lyngsøe 1962). Glucose concentration in the serum prepared for the SILA deter

[1] The insulin was supplied through the courtesy of Dr H. C. Hagedorn, M. D., Nordisk Insulin Laboratorium.

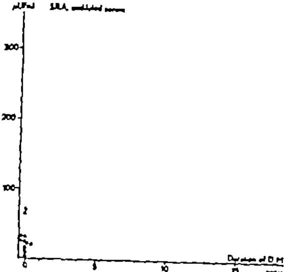


Fig 1 Relation between SILA in undiluted fasting serum and the duration of diabetes, non-obese diabetics.

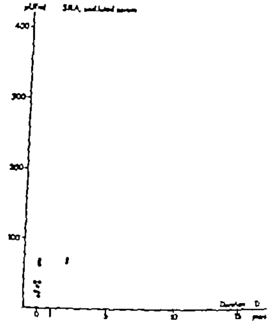


Fig 2. Relation between SILA in undiluted serum after glucose ingestion and the duration of diabetes, non-obese diabetics.

mination is determined by the glucose oxidase method (Hugget & Nixon 1957) Hagedorn-Normar Jensen's method (Hagedorn-Norman Jensen 1918) was used for the blood sugar determination to indicate the 24-hour level. The total CO_2 in the blood was determined by van Slyke's method (van Slyke et al 1921) The urine sugar level was determined by a reduction method using potassium ferricyanide

The material examined consists of a series of 55 patients suffering from d abetes, but not exhibiting acidosis (tabl I) All but three of the patients were examined both fasting and after glucose administration, while the three were examined only fasting The 52 patients who were fully examined provided the SILA values for statistical analysis.

The patients were assigned to *three groups* (table II) The first group consists of 16 patients whose diabetes mellitus was diagnosed before the age of 40 years. Only two of these patients are obese (Nos. 162 nd 194) The second group consists of 19 patients of normal weight, whose diabetes mellitus was diagnosed after the age of 40 years. The third group consists of 17 obese diabetics. In these patients the disease was diagnosed after the age of 40 years, and they were all obese at the time of examination Obesity is defined as a weight $\gtrsim$ 115 of the ideal weight obtained from the Hafnia" table of mean weights for a normal Danish population aged 30—34 years (Lyngsoe 1962)

Results

Comparing the SILA values for the three groups of diabetics no statistically significant difference was found between the values in the first two groups, which with two exceptions represent patients of normal weight In the third group, the obese diabetics the SILA levels showed statistically significantly higher values, for both undiluted and diluted serum than the other two groups (table III) In the following the SILA values for the first two groups the juvenile diabetics and the non-obese older dia betics, respectively are treated as be longing to one group non-obese dia betics As was to be expected from the above, the SILA values for the com bined group are significantly lower than the values for the group of obese diabetics (table III)

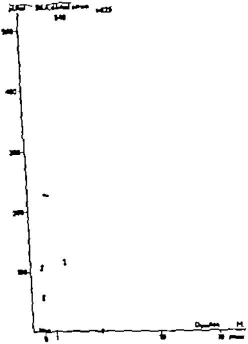


Fig. 3. Relation between SILA in diluted fasting serum and duration of diabetes, non-obese diabetics.

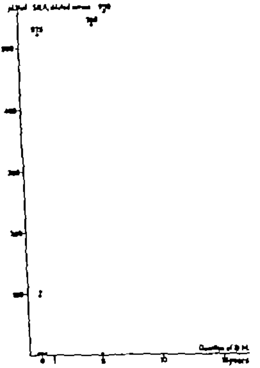


Fig. 4. Relation between SILA in diluted serum after glucose ingestion and the duration of diabetes, non-obese diabetics.

Other possible groupings of the patients were examined, to determine whether there were differences in the mean SILA values.

The *non-obese diabetics* (group 1 and 2) were divided into two subgroups, consisting of patients in whom diabetes mellitus had been diagnosed before and after the age of 25 years, respectively No statistically significant difference in SILA values of these groups could be demonstrated (table IV) nor was there any statistically significant difference in SILA values between non-obese diabetics receiving insulin treatment and patients on diet alone nor between patients receiving large and small insulin doses, respectively (table IV) Six non-obese diabetics had ketonuria, but not severe acidosis (patients Nos. 99 152, 192, 249 251 250) The SILA values in these patients corresponded to the fasting values in the rest of the non-obese diabetics.

Six patients with newly diagnosed diabetes were examined before starting insulin treatment. Their values corresponded to the fasting values in the remaining material of non-obese diabetics (table V) However if all the non-obese diabetics are divided into two groups, those with a duration of less than 6 months and those with a duration of more than 6 months statistically higher SILA values in undiluted serum can be demonstrated in the patients whose diabetes has been of long standing, as compared with the values for recent cases. A similar tendency seems to be

Table III Classification of the diabetic patients

	No.	SILA, undiluted serum μU/ml		SILA, diluted serum μU/ml	
		Fasting	After glucose	Fasting	After glucose
Obese diabetics	17	167.5 ± 9	300.7 ± 238	386.2 ± 245	618.2 ± 379
Juvenile diabetics	16	54.7 ± 47	85.6 ± 72	169.9 ± 172	233.1 ± 245
		t = 4.191 p < 0.001	t = 3.417 p < 0.01	t = 2.676 p < 0.02	t = 3.593 p < 0.01
Obese diabetics	17	167.5 ± 93	300.7 ± 238	386.2 ± 245	618.2 ± 379
Old, non-obese diabetics	19	95.1 ± 87	138.9 ± 93	178.2 ± 150	214.5 ± 178
		t = 2.578 p < 0.05	t = 2.739 p < 0.02	t = 3.131 p < 0.01	t = 4.171 p < 0.001
Juvenile diabetics	16	54.7 ± 47	85.6 ± 72	169.9 ± 172	233.1 ± 245
Old, non-obese diabetics	19	95.1 ± 87	138.9 ± 93	178.[illegible] ± 150	214.5 ± 178
		t = 1.660 p > 0.1	t = 1.874 0.1 > p > 0.05	t = 0.153 p > 0.1	t = 0.261 p > 0.1
Obese diabetics	17	167.5 ± 9	300.7 ± 238	386.2 ± 245	618.2 ± 379
Non-obese diabetics	35	76.7 ± 73	114.6 ± 87	174.3 ± 158	223.1 ± 207
		t = 3.786 p < 0.001	t = 4.114 p < 0.001	t = 3.792 p < 0.001	t = 4.877 p < 0.001

present in diluted serum but the difference between the two mean values is not statistically significant

Thus in the present material of non obese diabetics, no correlation was found between the fasting value of SILA and the age at which diabetes was diagnosed, or between the fasting value of SILA and the degree of diabetes as expressed by the dose of insulin the SILA values in patients with slight ketosis were neither strikingly high nor low On the other hand, the SILA values during the first few months of diabetes appear to be somewhat lower than later in the course of the disease.

In the group of *obese diabetics* no statistically significant difference could be found between the SILA values in patients having insulin treatment and those not having insulin treatment (table IV) Nor could any difference be demonstrated in this group between SILA values from patients with varying duration o diabetes.

In neither of the two groups of diabetics, non-obese (younger and older patients) and obese (older patients) could any relation be demonstrated between the fasting SILA values and the fasting serum glucose values at the time of blood sampling (figs. 5—8)

After *glucose ingestion*, the mean figures for SILA were found to be higher than the corresponding mean figures for the fasting values, both for the group of non-obese diabetics and for the group of obese diabetics (table III) The obese diabetics show significantly higher SILA values after glucose ingestion than in the fasting state, for both undiluted and diluted serum. In the case of undiluted serum, the non-obese diabetics show a statistically significant increase in SILA

Table IV Classification of non-obese diabetics

	No.	SILA, undiluted serum μU/ml		SILA, diluted serum μU/ml	
		Fasting	After glucose	Fasting	After glucose
Onset before age 25 yrs.	7	41.9 ± 32	68.1 ± 65	121 ± 78	150.7 ± 92
Onset after age 25 yrs.	28	85.4 ± 78	126.1 ± 88	187.6 ± 171	241.1 ± 225
		t = 1.426 $p > 0.1$	t = 1.619 $p > 0.1$	t = 0.990 $p > 0.1$	t = 1.032 $p > 0.1$
Patients with insulin requirement	24	73.8 ± 72	101.6 ± 77	175.8 ± 153	239.0 ± 231
Patients without insulin requirement	11	83.2 ± 80	142.7 ± 113	171.4 ± 176	188.2 ± 147
		t = 0.309 $p > 0.1$	t = 1.263 $p > 0.1$	t = 0.075 $p > 0.1$	t = 0.667 $p > 0.1$
Patients receiving more than 20 i.u./24 hrs.	17	54.4 ± 43	89.9 ± 72	140.5 ± 144	215 ± 223
Patients receiving 20 or less i.u./24 hrs.	18	97.7 ± 90	157.8 ± 95	206.4 ± 168	230.6 ± 190
		t = 1.862 $0.1 > p > 0.05$	t = 1.673 $p > 0.1$	t = 1.241 $p > 0.1$	t = 0.219 $p > 0.1$
Duration of diabetes ≤ 6 months	15	43.7 ± 36	80.6 ± 74	140.9 ± 137	173.3 ± 165
Duration of diabetes > 6 months	20	101.4 ± 84	140.0 ± 89	199.5 ± 171	260.3 ± 252
		t = 2.598 $0.02 > p > 0.01$	t = 2.542 $0.02 > p > 0.01$	t = 0.901 $p > 0.1$	t = 1.257 $p > 0.1$
Classification of obese diabetics					
Patients on insulin	6	168.3 ± 73	397.8 ± 358	437.5 ± 255	650.0 ± 453
Patients not on insulin	11	167.1 ± 110	247.7 ± 134	358.2 ± 244	600.9 ± 354
		t = 0.023 $p > 0.1$	t = 1.264 $p > 0.1$	t = 0.650 $p > 0.1$	t = 0.248 $p > 0.1$

Table V Newly-diagnosed, juvenile diabetics

Assay no.	Patient no.	Age	Serum-glucose mg %	SILA fasting		Lambda	No. of rats
				Undiluted μU/ml	Diluted μU/ml		
203	93	22	185	23	160	0.23	3
235	135	23	329	24	0	0.50	4
251	193	15	201	70	110	0.39	4
317	249	16	237	56	165	0.34	4
323	250	23	180	132	875	0.15	4
358	251	22	208	175	220	0.24	4
			Mean	80	255		

Table III Classification of the diabetic patients

	No.	SILA, undiluted serum μU/ml		SILA, diluted serum μU/ml	
		Fasting	After glucose	Fasting	After glucose
Obese diabetics	17	167.5 ± 95	300 7 ± 238	386.2 ± 245	618.2 ± 379
Juvenile diabetics	16	54 7 ± 47	85.6 ± 72	169.9 ± 172	233 1 ± 245
		t = 4.191 p < 0.001	t = 3.417 p < 0.01	t = 2.676 p < 0.02	t = 3.393 p < 0 01
Obese diabetics	17	167.5 ± 95	300.7 ± 238	386.2 ± 245	618.2 ± 379
Old, non-obese diabetics	19	95.1 ± 87	138.9 ± 93	178.2 ± 150	214.5 ± 178
		t = 2.378 p < 0.05	t = 2.739 p < 0 02	t = 3.131 p < 0.01	t = 4.171 p < 0.001
Juvenile diabetics	16	54.7 ± 47	85 6 ± 72	169.9 ± 172	233.1 ± 245
Old, non-obese diabetics	19	95.1 ± 87	138.9 ± 93	178.2 ± 150	214.5 ± 178
		t = 1 660 p > 0.1	t = 1.874 0.1 > p > 0.05	t = 0.155 p > 0.1	t = 0.261 p > 0.1
Obese diabetics	17	167 5 ± 95	300.7 ± 238	386.2 ± 245	618.2 ± 379
Non-obese diabetics	35	76.7 ± 73	114.6 ± 87	174.3 ± 158	223.1 ± 207
		t = 3.786 p < 0.001	t = 4.114 p < 0.001	t = 3.792 p < 0.001	t = 4.877 p < 0.001

present in diluted serum, but the difference between the two mean values is not statistically significant.

Thus, in the present material of non obese diabetics no correlation was found between the fasting value of SILA and the age at which diabetes was diagnosed, or between the fasting value of SILA and the degree of diabetes as expressed by the dose of insulin the SILA values in patients with slight ketosis were neither strikingly high nor low On the other hand, the SILA values during the first few months of diabetes appear to be somewhat lower than later in the course of the disease.

In the group of *obese diabetics* no statistically significant difference could be found between the SILA values in patients having insulin treatment and those not having insulin treatment (table IV) Nor could any difference be demonstrated in this group between SILA values from patients with varying duration o diabetes

In neither of the two groups of diabetics, non-obese (younger and older patients) and obese (older patients) could any relation be demonstrated between the fasting SILA values and the fasting serum glucose values at the time of blood sampling (figs 5—8)

After *glucose ingestion*, the mean figures for SILA were found to be higher than the corresponding mean figures for the fasting values, both for the group of non-obese diabetics and for the group of obese diabetics (table III) The obese diabetics show significantly higher SILA values after glucose ingestion than in the fasting state, for both undiluted and diluted serum. In the case of undiluted serum, the non-obese diabetics show a statistically significant increase in SILA

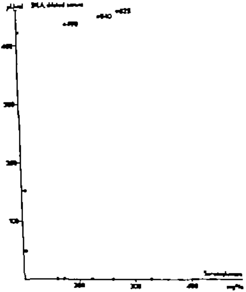


Fig. 6. Relation between serum glucose and SILA in diluted fasting serum from non-obese diabetics.

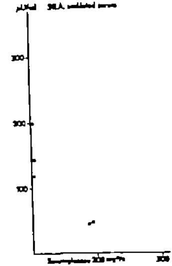


Fig. 7. Relation between serum glucose and SILA in undiluted fasting serum from obese diabetics.

Table VI. The SILA increase after glucose administration

	No.	SILA	
		Undiluted µU/ml	Diluted µU/ml
Non-obese diabetics	35	37.9 ± 3.5 t = 4.031 p < 0.001	48.8 ± 211 t = 1.366 p > 0.1
Obese diabetics	17	153.2 ± 22.4 – 2.591 p 0.02	232 ± 34.3 t = 2.788 p < 0.02

fasting serum show no correlation (fig. 11) while in serum drawn after glucose ingestion, higher SILA values in diluted serum are found to correspond to higher SILA values in undil ted serum (fig 12).

The regression coefficients expressing the relation between SILA in diluted and undiluted serum were not significantly different in normals (Lyngsoe 1962) and diabetics (table IX)

Discussion

The question whether diabetes mellitus can be divided into two or more types" which may be different diseases, has been discussed for almost a century

Our studies have shown that diabetics can be divided into two groups on the basis of the SILA values. In the first group patients developing diabetes mellitus before the age of 40 years and non-obese patients developing diabetes mellitus after the age of 40 years normal average SILA values were found. In the second group older obese diabetics, the average SILA values were elevated.

Other investigators have obtained results deviating in some respects from ours, possibly to a certain extent on account of differences in the methods of investigation.

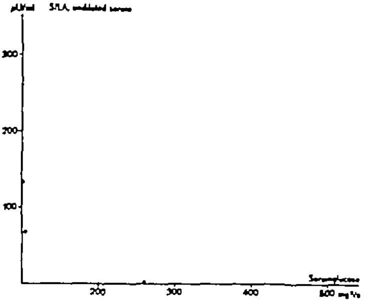


Fig. 5. Relation between serum glucose and SILA in undiluted fasting serum from non-obese diabetics.

values after glucose ingestion, while the average increase in diluted serum, though of the same order of magnitude, was statistically non-significant (table VI)

The SILA values in the present material of diabetics were compared with the values in a normal material examined under the same experimental conditions (Lyngsoe 1962) The SILA values for the non-obese diabetics are found to be of the same order of mag nitude as normal values, both fasting and after ingestion of glucose (table VII) After glucose ingestion diluted serum from normal subjects shows a tendency to higher SILA values than diluted serum from the non-obese diabetics but the difference is not statistically signifi cant ($0.1 > p > 0.05$) On the other hand, as was to be expected from what has been stated above, the SILA values for the group of obese diabetics were higher than those for the normal material, both fasting and after ingestion of glucose. The mean values for the *rise in SILA level* following glucose ingestion show a similar tendency but the difference is not statistically significant (table VII)

Figs 9—12 show the relation between the SILA values in undiluted and diluted serum from the individual patients. In most of the sera from both obese and non-obese diabetic patients, the SILA values in diluted serum are seen to be higher than the corresponding values in undiluted serum. If the dilution effect is calculated as the difference between the SILA values in diluted and undiluted serum, it can be shown that this figure is significantly greater than zero in both groups of diabetics (table VIII)

The group of non-obese diabetics shows some degree of correlation between the SILA values in undiluted and diluted serum. In fasting serum, however the correlation can only be shown in the range between 0 and 140 µU/ml while in serum samples taken after glucose ingestion the values are correlated over the whole range from 0—380 µU/ml (figs. 9—10)

In the group of obese diabetics the SILA values in undiluted and diluted

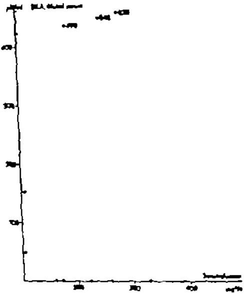


Fig. 6. Relation between serum glucose and SILA in diluted fasting serum from non-obese diabetics.

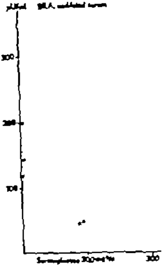


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Obese diabetics	17	153.2 ± 22.4 t = 2.591 p < 0.02	232 ± 34.3 t = 2.788 p < 0.02

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The regression coefficients expressing the relation between SILA in diluted and undiluted serum were not significantly different in normals (Lyngsoe 1962) and diabetics (table IX)

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Other investigators have obtained results deviating in some respects from ours, possibly to a certain extent on account of differences in the methods of investigation.

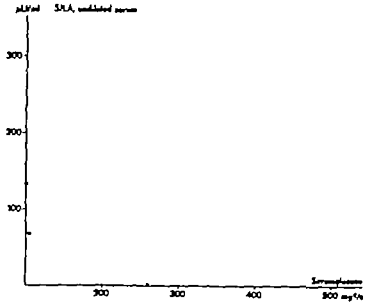


Fig 5. Relation between serum glucose and SILA in undiluted fasting serum from non-obese diabetics.

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In the group of obese diabetics the SILA values in undiluted and diluted

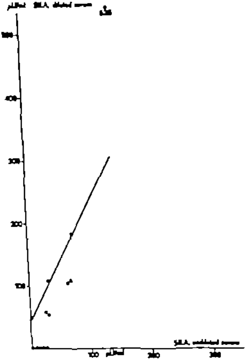


Fig. 9. Relation between SILA in undiluted and diluted fasting serum from non-obese diabetics.
b_{yx} 1.060 $s_{b_{yx}}$ = 0.563 t = 3.303 p = 0.001

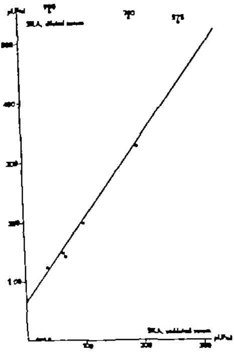


Fig. 10. Relation between SILA in undiluted and diluted serum after glucose ingestion in non-obese diabetics.
$b_{y.x}$ = 1.415 $s_{b_{y}}$ = 0.358 t = 3.952 p < 0.001

Willebrands et al. (1956) determined SILA in diluted serum from four juvenile diabetics by means of the rat diaphragm method. It was possible to demonstrate insulin-like activity in these four patients, but the study provides no information on the regulation of the diabetes at the time of examination. Investigators working with the rat-epididymal fat method have obtained results which differ from those obtained with the rat diaphragm method. Pfeiffer et al. (1959) using the rat epididymal fat method, found insulin-like activity in diluted serum from four juvenile diabetics. Steinke et al. (1961) have published a major series of SILA determinations obtained by the same procedure. In undiluted serum from 28 untreated juvenile diabetics, they found fasting SILA values which were somewhat higher than those of their normal material.

In the present study insulin-like activity was found in undiluted and diluted serum from juvenile diabetics, both those who were well-regulated and those with a slight ketosis. The SILA values did not differ from those found in normal experimental subjects, either in fasting determinations or after ingestion of glucose. This is in contrast to the findings of Steinke et al., but it is not clear whether the composition of the latter material differs from that of our own. In Steinke et al.'s material, information is lacking on the age of the patients, the duration of the disease and the degree of acidosis.

Table VII Comparison of SILA values in diabetic subjects and normal material

	No.	SILA, undiluted serum $\mu U/ml$			No.	SILA, diluted serum $\mu U/ml$		
		Fasting	After glucose	Increase		Fasting	After glucose	Increase
Non-obese diabetics	35	76.7 ± 73	114.6 ± 87	37.9 ± 55	35	174.3 ± 158	223.1 ± 207	48.8 ± 2
Normal persons	23	62.3 ± 38	135.7 ± 117	73.4 ± 110	22	169.6 ± 138	320.6 ± 21	151.0 ± 1
		$t = 0.862$ $p > 0.1$	$t = 0.770$ $p > 0.1$	$t = 1.599$ $p > 0.1$		$t = 0.114$ $p > 0.1$	$t = 1.699$ $0.1 > p > 0.05$	$t = 1.917$ $0.1 > p > 0.$
Obese diabetics	17	167.5 ± 95	300.7 ± 238	133.2 ± 224	17	386.2 ± 245	618.2 ± 379	232.0 ± 3
Normal persons	23	62.3 ± 38	135.7 ± 117	73.4 ± 110	22	169.6 ± 138	320.6 ± 212	151.0 ± 16
		$t = 4.814$ $p < 0.001$	$t = .853$ $p < 0.01$	$t = 1.106$ $p > 0.1$		$t = 3.403$ $p < 0.01$	$t = 3.091$ $p < 0.01$	$t = 0.971$ $p > 0.1$

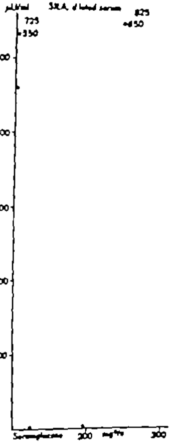


Fig. 8. Relation between serum glucose and SILA in diluted fasting serum from obese diabetics.

Table VIII The dilution effect

Fasting serum

	Mean $\mu U/ml$	Standard dev.	t	p
Non-obese diabetics	97.6	139	4.155	< 0.00
Obese diabetics	218.6	223	4.048	< 0.001

Serum after glucose ingestion

	Mean $\mu U/ml$	Standard dev.	t	p
Non-obese diabetics	108.5	183	3.500	< 0.01
Obese diabetics	317.5	295	4.434	< 0.001

Bornstein et al in 1951 using the ADHA rat method, found that the serum of diabetics with ketosis was devoid of insulin-like activity However once the diabetic metabolic anomaly was regulated by insulin treatment, SILA could be demonstrated in these patients. Vallance Owen et al. (1955) and Seltzer & Smith (1959) using the rat diaphragm method arrived at the same results by studying undiluted blood from poorly-regulated non ketotic juvenile diabetics.

inhibits the effect of insulin on the glucose uptake of the rat diaphragm. A simple extraction method made it possible later to isolate the inhibiting factor "insulin antagonist" It seems probable that the presence of this insulin antagonist is the cause of SILA not being demonstrated in undiluted serum from poorly regulated juvenile diabetics, using the rat diaphragm method. Recent studies using the rat epididymal fat method have been unable to demonstrate a corresponding inhibition of insulin, added to serum from this group of diabetics (Lyngsøe, in preparation) just as the isolated antagonist does not appear to be active on fatty tissue (Lowy et al. 1961 Lyngsøe in preparation) It thus appears as if the Vallance-Owen insulin antagonist is active when rat muscle is used as test object, but inactive on rat fat. This phenomenon may explain the discrepancies between the results obtained in determinations of SILA in juvenile diabetics, using the rat diaphragm method and the rat epididymal fat method, respectively

Analyzing our results for older diabetics, it was found of importance to distinguish between obese and non-obese patients. In serum from non-obese diabetics in whom diabetes mellitus was diagnosed after the age of 40 we found SILA values which did not differ from the values in juvenile diabetics and normal subjects. Only eight out of these 19 older non-obese diabetics were under insulin treatment, so that in the usual clinical sense, a considerable difference exists between the patients in this group and the juvenile diabetics. In spite of this, we found the same average SILA values.

In the literature, the only study containing information on SILA in older non-obese diabetics is by Seltzer & Smith (1959) Using the rat diaphragm method they examined a group of diabetics corresponding in weight and age to our group of older non-obese diabetics, but these authors do not state the age of onset. In the investigation cited, the patients are divided into "Tolbutamide responders" and "Non-Tolbutamide responders" However if the mean SILA value for these two groups, excluding one patient who was overweight, is calculated all in one, it is found that there is no significant difference between this mean value and the mean value in Seltzer's normal material.

There are several studies of SILA in older obese diabetics (Bornstein et al. 1951 Vallance-Owen et al. 1955 Rizzo et al. 1961) SILA was demonstrated in these patients in all studies cited. Bornstein using the ADHA rat method, found lower values than in his normal material, when determinations were made two hours after glucose. But the patients were not examined fasting. Vallance-Owen et al. using the rat diaphragm method, found fasting values that were higher than those in a normal material, while the SILA values after glucose ingestion corresponded to normal values. Rizzo et al. examining diluted serum by the rat diaphragm method, found the same fasting SILA values in older obese diabetics as in normal subjects. In the present study higher SILA values were found in older obese diabetics than in normal subjects, both fasting and after glucose ingestion, and after glucose ingestion the rise in SILA was of the same order of magnitude as in normal subjects. It is possible that the reason for this lack of agreement in the results between Vallance-Owen et al. and the present author is that the patients were examined

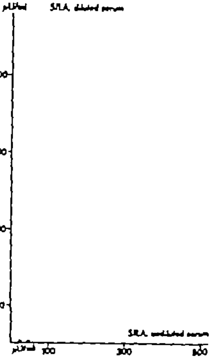


Fig 11 Relation between SILA in undiluted and diluted fasting serum from obese diabetics.
$y.x = 1\ 000\ e_{b_y} = 0.603\ t = 1\ 638\ p > 0.1$

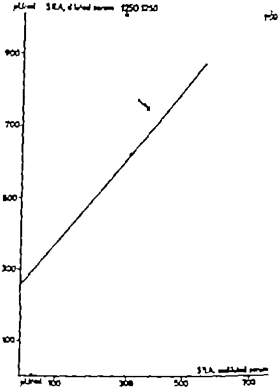


Fig. 12 Relation between SILA in undiluted and diluted serum after glucose ingestion in obese diabetics.
$b_y = 0.995\ e_{b_y} = 0.320\ t = 3\ 109\ p < 0.01$

The decisive feature, however is that like Pfeiffer et al. and Steinke et al., we were able to show that serum from poorly regulated juvenile diabetics possesses insulin like activity as examined by the rat epididymal fat method. As mentioned Vallance-Owen and Seltzer reach the opposite result using the rat diaphragm method. It is unlikely that this is because their patients were more poorly regulated than ours. In a series of later studies with the rat diaphragm method, Vallance-Owen et al. (1958) have shown however that *in vitro* serum from poorly regulated juvenile diabetics

Table IX. The regression coefficients for the relation between SILA in undiluted and diluted serum, compared between normal subjects and diabetics

	Fasting serum			Serum after glucose		
	b_y	e_{b_y}	No.	b_y	e_{b_y}	No.
Normal	0.915	0.435	73	0.915	0.318	22
Non-obese diabetics	1.860	0.563	35	1 415	0.358	35
	$t = 1.329$	—	—	$t = 1.045$	—	—
	$p > 0.1$	—	—	$p > 0.1$	—	—
Obese diabetics	—	—	—	0.995	0.320	17
	—	—	—	$t = 0.176$	—	—
Normal ~ obese diabetics	—	—	—	$p > 0.1$	—	—

in blood sugar there is a pronounced elevation in SILA, measured by the rat diaphragm method. Thus, in cases of juvenile diabetes and in older non-obese diabetics, where normal values are found for SILA along with elevated blood sugar these values, seen in relation to the serum glucose values, may be regarded as abnormally low This might be regarded as being in accordance with the view that these patients have a reduced insulin production, but it might also be taken to indicate that part of the insulin in the serum from these patients is present in an inactive state, with respect to the tissue used for measuring SILA.

The high SILA values in the group of older obese diabetics may be regarded as a normal response to the elevated serum glucose. However it is not known whether the SILA values observed are of the same order of magnitude as they would be in normal subjects who by glucose infusion, have been maintained for some time on correspondingly high serum glucose values. The possibility cannot be excluded, therefore, that actually the SILA values also in this group of diabetics are reduced just as in the non-obese group

Ehrlich and Randle (1961) have recently demonstrated an elevated level of growth hormone in blood from obese patients, and Vallance-Owen & Lilley (1961) have been able to show that in these patients the serum has a greater content than in normals of the pituitary-dependent insulin antagonist described by Vallance-Owen. It is therefore possible that the activity of insulin in serum from obese diabetics is inhibited by the insulin antagonist, and that this is the cause of the diabetes in these patients. It is also possible that the increased amount of growth hormone present in obese diabetics by itself causes the cells in the peripheral tissues to develop reduced insulin sensitivity Galbraith et al. (1961) have recently been able to show that following the injection of growth hormone, the tissues in the forearm of a normal subject show a reduced glucose uptake, and a reduced response to insulin. Although opinions differ as to the action of growth hormone on the uptake of glucose and the effect of insulin on muscle tissues (Krahl 1952, Ottaway 1953 Manchester et al. 1959) the theory of a reduced peripheral insulin-sensitivity should be regarded as a reasonable possibility

Finally the possibility should be considered that the explanation of the diabetes in older obese patients, along with elevated SILA values, might be that the SILA values express insulin which is in some way different from normal insulin, for example by having a different form of protein linkage. It has been shown in the present study that when serum from diabetics is diluted, there is a rise in the SILA value, as in normal subjects. If this dilution effect is an expression of a release of protein-bound insulin, which can be measured by the rat epididymal fat method, this does not support the hypothesis that the binding of insulin to the plasma proteins in older obese diabetics differs in nature from that in normal subjects.

Summary

An examination of the SILA in 55 diabetics is reported. SILA has been determined by the rat epididymal fat method in both undiluted and diluted (20 %) serum, and has been measured fasting as well as 1 ½ hour after ingestion of glucose.

one hour after glucose ingestion in Vallance Owen et al s study as against $1^1/_2$ hours in the present study There are no determinations of SILA in older obese diabetics at different intervals after glucose ingestion but Yalow & Berson s (1960) study of newly-diagnosed patients suffering from "mild diabetes seems to suggest that SILA, determined immunologically rises more slowly in such patients than in normal subjects.

In most studies of SILA in older diabetics information is lacking on the weight of the patients (Yalow & Berson 1960 Steinke et al. 1961 Pfeiffer et al 1961) These studies, therefore do not help to elucidate the difference in SILA in older obese and non-obese diabetics demonstrated in the present study Pfeiffer at al. (1961) found that grouping of older diabetics according to the presence or absence of response in SILA values to ingestion of glucose accorded well with the clinical grouping in mild and somewhat more severe cases the first group required dietary treatment only but the second group needed dietary treatment plus a peroral antidiabetic drug In the present study a division according to the response to glucose ingestion did not show any clinical difference between the two groups.

The present study *has shown that in* undiluted serum from non-obese diabetics, the SILA values in patients who had a *duration of diabetes* of more than six months were higher than those in patients with a shorter duration of diabetes We are unable to explain this It might be due to a greater amount of exogenous or endogenous insulin in the serum. We have tried to avoid the presence of exogenous insulin in serum used for SILA determinations by drawing blood 24 hours after the last dose of insulin Studies with I^{131}-labelled insulin in normal subjects suggest that after intravenous injection of insulin only 8 % remains in the plasma one hour after the injection However patients on insulin treatment for more than four months have been shown to retain a larger amount of insulin in the plasma, as the insulin here is bound to antibodies (Welsh et al. 1955) Thus it is conceivable that the high insulin like activity in undiluted serum from non-obese patients with diabetes of fairly long standing could be due to exogenous insulin However if these patients are divided into two groups, those having insulin treatment for more than six months and the other with patients not having insulin treatment, the SILA in undiluted serum is found to be of the same order of magnitude in both groups. It must therefore be considered improbable that the high SILA values in these patients are due to exogenous insulin.

It might appear remarkable that, in the present study serum from juvenile and from older non-obese diabetics showed SILA values which were of the same order of magnitude as in our normal material However the SILA values probably should be evaluated in relation to the serum glucose values. In *the dog Metz (1960) has shown* that measured by the rat diaphragm method, using blood from the pancreaticoduodenal vein the production of insulin is proportional to the serum glucose concentration It must be assumed, therefore that with elevated serum glucose values, the peripheral venous blood will also show high SILA values. This has been confirmed recently as Seltzer (1961) has shown that with continuous intravenous infusion of glucose to normal subjects, in spite of only a slight rise

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The Immune-Globulins in Mongolism

By

BENGT SKANSE and CARL BERTIL LAURELL

In the course of our routine work serum from a patient with mongolism was found to have a gamma globulin content as high as 6.6 g per 100 ml[1]. This observation prompted us to study the serum protein fractions in mongolism. Perusal of the literature revealed that in mongolism the gamma globulins are increased, that the albumin is decreased (Stern & Lewis 1957, Sobel et al. 1958, Carver et al. 1959, Nelson 1961) and that ultracentrifugal analyses and studies of the amino acid composition of the gamma globulins from mongoloids have shown no qualitative abnormalities (Carver & Welton 1961). But no reports on the immune-globulins β_{2A} and β_{2M}[2] in mongoloids could be traced.

Material and methods

The material consisted of 25 mongols, aged 15 to 48 years, selected at random from two mental homes. None of the patients presented any signs of infections, inflammatory reactions, liver disease, malnutrition or neoplastic disease.

Paper electrophoresis was performed in accordance with Laurell et al. (1956). The haptoglobin concentration was determined by a modified Jayle technique (Laurell & Grönvall). The micromethod of Scheidegger (1955) as modified by Heremans (1960) was applied for immunoelectrophoretic analyses. Rabbit antisera of different specificity (anti-γ, anti-β_{2A} and anti-β_{2M}) were used in the study of the immune-globulins. The serum cholesterol was measured by the method of Klungsöyr et al. (1958).

Results

The results of the quantitative serum protein analyses are summarized in fig. 1 which gives all individual data in relation to age.

The concentration of total serum proteins was usually normal but slightly increased in 3 patients. The albumin concentration was also normal, except in 3 patients, in whom it was slightly decreased. The β_1-globulin concentration was decreased in 7 patients and the β_2-globulin concentration was increased,

[1] Later this patient was found to have systemic lupus erythematosus and is not included in the present material.

[2] Synonyms γ_{1A} and γ_{1M}, respectively.

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The diabetic patients have been divided into two groups one consisting of obese patients with diabetes diagnosed after the age of 40 the other consisting of non-obese diabetics The older obese diabetics had higher SILA values than the non-obese, both in undiluted and diluted serum. The difference is statistically significant

The SILA values for the older obese diabetics were higher than the normal values while no statistically significant difference could be demonstrated between the values in non-obese diabetics and normal subjects.

Following glucose ingestion a statistically significant rise in SILA activity could be demonstrated in undiluted and diluted serum from older obese diabetics On the other hand, in non-obese diabetics a significant rise in SILA values could be demonstrated in undiluted serum but not in diluted serum.

In non-obese patients with diabetes of more than six months duration undiluted serum showed a tendency to SILA values higher than in patients with a shorter duration of diabetes.

In serum from both groups of diabetics, higher SILA values were found in diluted than in undiluted serum. The correlation between SILA values in undiluted and diluted serum was the same in both groups of diabetics, and of the same order of magnitude as in normal subjects.

Acknowledgement

I wish to express my gratitude to Miss Tove Dreyer Petersen for skilled technical assistance

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globulinemia may be inherited (Leonhardt 1957) Whether this may be directly referred to differences in the genetic material is not yet known, but the disturbance in chromosome number 21 or other chromosomal alterations in mongoloids may be of significance in this respect.

Our immunoelectrophoretic studies almost invariably showed an increase of β_{2A}-globulins. Though no method for quantitative measurment of β_{2A} or β_{2M} globulins is available, good agreement was found between the β_{2A}-globulins and paper electrophoretic analyses. The β_{2M} is more difficult to assess but it tended to vary with the other two fractions. It would thus appear that all the three main types of immune-globulins had reacted in the common way to increased antigenic stimulation.

Carver et al. (1959) and Nelson (1961) found the concentration of the gamma globulins to increase with age in mongolism. This could not be confirmed in the present study (fig 1) The discrepancy may be due to our patients being older their ages ranging from 13 to 48 years by which time the normal increase of the gamma globulin concentration during childhood was probably completed despite the known delay in mental development (Stern & Lewis 1957)

The common occurrence of increase of the electrophoretic β_1-globulin fraction was not accompanied by any increase of the cholesterol bound to this globulin fraction. The increase of the slow beta globulins is probably of the same origin as the increase of the gamma globulins. Nelson (1961) observed an increase in the amount of lipoprotein in the beta fraction in mongoloids without any accompanying change in the β-globulins (β_1 and β were not separated)

The clinical implication of the present observation is that mongolism per se may be responsible for elevation of the gamma globulins and that it is therefore probably not necessary to search for any underlying chronic process.

Summary

Increased gamma globulin concentrations were observed on paper electrophoresis of sera from 22 of 25 patients with mongolism. The albumin concentration was normal.

Immunoelectrophoretic studies revealed normal quantitative co-variation of the immune-globulins (γ β_{2A} and β_{2M})

Acknowledgment

The authors are greatly indebted to Dr Edvin Persson for placing the blood samples at their disposal.

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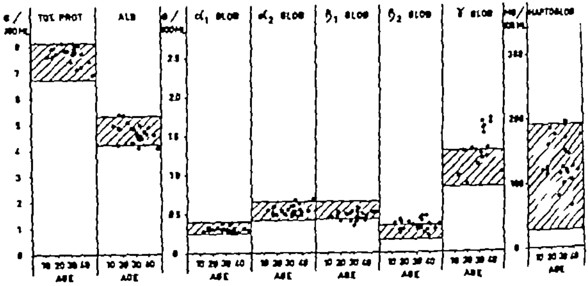


Fig 1 Distribution of serum protein fractions in mongolism in relation to age

though only slightly in 12 patients. The gamma globulin concentration was increased in 14 patients, the highest value noted being 2 43 g per 100 ml The gamma globulin increase was always of a diffuse type In no case was there any sign of any M-components.

The haptoglobin concentration was significantly increased in one patient. This patient also had the highest α_2-globulin value (0 75 g per 100 ml) and also the highest gamma globulin value (2 43 g per 100 ml) These findings suggested the possibility of some additional abnormality

None of the serum protein fractions studied were found to vary with the patients age.

The immunoelectrophoretic findings were in full accord with the paper electrophoretic pattern, i. e. the precipitation γ-lines being of intensity corresponding to normal or increased γ-globulin concentrations. The β_{1A}-globulin level was found to be elevated in 22 of the 25 patients, while that of the β_{1M}-globulins appeared to be essentially normal.

The β-lipoprotein cholesterol varied between 114 and 278 mg per 100 ml (average 196 mg per 100 ml) and was thus largely normal

Discussion

Observations made in the present investigation thus endorsed the existence of a raised concentration of gamma globulins in mongolism but failed to confirm any depression of the albumin concentration.

The cause of the gamma globulin increase is obscure, for the patients showed no evidence of diseases known to raise the gamma globulin level The largely normal concentrations of the a globulins and haptoglobins also weigh heavy against the existence of any active process in any of these patients except one. Isolated elevation of the gamma globulins may be observed even in quiescent chronic processes, but all the subjects studied in the present investigation appeared to be "healthy"

Factors predisposing to hypergamma-

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Studies on Glycolysis in Hereditary Non-spherocytic Hemolytic Disease

By

Arne Nordøy Jon Bremer, Erik Myhre and Svein Solheim

Hereditary non-spherocytic hemolytic anemia is a relatively uncommon disease due to an intracellular defect of the red cells (2) This disease differs from hereditary spherocytosis by a normal morphology of the red cells and a normal osmotic fragility Splenectomy is usually without influence on the condition. Based upon differences of the autohemolysis of the red cells during incubation at 37 C, Dacie et al. (1) and Selwyn and Dacie (3) differentiated between two types of this disorder In type I the autohemolysis is normal or nearly normal, and the hemolysis is partially inhibited by glucose. In type II there is a markedly increased autohemolysis which is not inhibited by glucose. The basic disorder in this disease was postulated to be a defective carbohydrate metabolism.

We have studied four cases of hereditary non-spherocytic hemolytic anemia. In two cases, which corresponded to type I we found a lack of glucose 6-phosphate dehydrogenase (G-6-PD) activity a reduced oxygen uptake in the presence of methylene blue, and a decreased disulfide reduction capacity in the red cells. In the other two cases, which fitted type II we found the glycolysis and the oxygen uptake to be within the normal range.

Methods

The oxygen uptake of erythrocytes was measured in the presence of methylene blue, and the reduction of the disulfide cysteamine was determined as previously described by Eldjarn et al. (29) In the oxygen uptake experiments, 0.5 ml packed red cells were incubated with 2.5 ml of the incubation medium in each Warburg flask. The disulfide reduction was measured by incubation of 1 ml packed red cells with 19 ml of incubation medium in the presence of 10^{-4} M cystamine.

Glucose 6-phosphate dehydrogenase (G-6-PD) activity was determined by measurement of the increase in optical density at 340 mμ in hemolysates after addition of glucose 6-phosphate and triphosphopyridine nucleotide (TPN)

Adenosine monophosphat (AMP) adenosine diphosphate (ADP) and adenosine tri-

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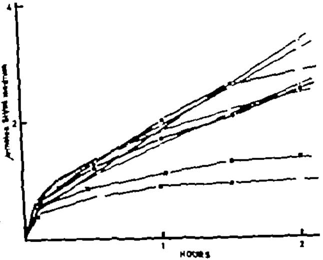


Fig. 2. Disulfide (cystamine) reduction by erythrocytes from four normal individuals and from four patients with hereditary non-spherocytic hemolytic anemia. Symbols as in fig. 1.

Case 3. A twenty-three-year-old male. At the age of three months anemia was diagnosed, and at four months he got jaundice and a slightly enlarged spleen. In 1953 splenectomy was performed. The removed spleen weighed 480 g, and it had a normal pattern. The clinical condition remained unchanged after the splenectomy. The most important laboratory findings obtained in 1959 are listed in table 1. The morphology of the red cells and the osmotic fragility were normal. The icterus index was 9, and the serum iron concentration 72 μg/100 ml. Coombs test was negative. The bone marrow was hypercellular with an increased percentage of normoblasts. On re-examination in September 1961 the condition was unchanged.

Case 4. A forty-eight-year-old female; she was the mother of case 3. She had for many years been anemic with hemoglobin value between 7.5 and 10.5 g/100 ml. She had never been icteric. The reticulocyte count was 1.3 per cent. The red cell morphology and the osmotic fragility were normal. The autohemolysis after 48 hours was 0.80 per cent, and addition of glucose raised it to 1.67 per cent.

In the first family thirteen members were examined, among whom cases 1 and 2 had a hemolytic disease. Only four members of the second family could be examined, the disease being found in cases 3 and 4. Only cases 1 and 3 were admitted to the hospital, the others being outpatients. Therefore cases 1 and 3 could be examined more thoroughly than the others.

Results

The cases of the first family, cases 1 and 2, could be classified as type I of hereditary non-spherocytic hemolytic anemia based upon the autohemolysis tests. Although the autohemolysis was only moderately increased in cases 3 and 4, the increase of hemolysis by addition of glucose proved that these patients belonged to type II of the disease. In both families mother and son were affected.

Fig. 1 shows the methylene blue-stimulated oxygen uptake of the red cells from the patients, and from four healthy individuals. It is evident that the four patients fall into two groups. Cases 1 and 2 (type I) showed a distinctly lower oxygen uptake than normal, whereas cases 3 and 4 (type II)

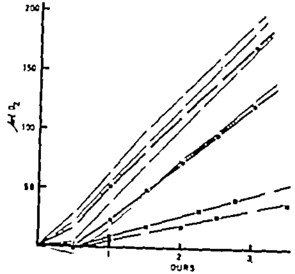


Fig 1 The methylene blue-stimulated oxygen uptake by erythrocytes from four normal individuals and from four patients with hereditary non-spherocytic hemolytic anemia.
— — = 4 randomly chosen normal individuals.
■ = case 1 = case 2
○ = case 3 ● = case 4

phosphate (ATP) were measured as described by Mills and Summer (4)

The life span of the patients own ⁵¹Cr labeled red cells was determined according to Mollison and Veall (5) and the data were corrected for a uniform ⁵¹Cr-elution after Hughes Jones and Mollison (6)

The red cell production was evaluated by the use of ⁵⁹Fe (7) and reticulocyte counts.

The red cell mass was measured with ⁵¹Cr labeled red cells (8) and the plasma volume by Evans blue and by measurement of the dilution of a known amount of protein bound ⁵⁹Fe injected intravenously

The autohemolysis was determined by the method of Selwyn and Dacie (3)

Case reports

Case 1 A thirty-seven-year-old male. He was moderately jaundiced since early child hood but otherwise healthy In 1951 he was severely anemic with a reticulocyte count of 28 per cent. The red cells showed a normal morphology and the osmotic fragility was normal. An X ray examination showed gallstones. Since that time he had been healthy except for a moderate jaundice.

Table 1 Hematological findings in the two patients

	Case 1 (Type 1)	Case 3 (Type II)	Normal values in our laboratory
Hemoglobin conc. (g/100 ml)	12.5	9.6	14–16
Erythrocytes (10^6/mm)	4.0	3.5	4.5–6.5
Reticulocytes (%)	8.5	11.8	0.5–1.7
T/2 ⁵¹Cr labeled erythrocytes (days)	7	8	24–31
Erythrocyte destruction (% of red cell mass per day)	6.4	5.6	0.8–1.0
Hemoglobin break down (g/day)	51	24	6–8
T/2 plasma ⁵⁹Fe (min.)	22	–	74–140
Incorporation of ⁵⁹Fe into erythrocytes (% of given dose)	96	–	75–95
Hemoglobin synthesis (g day)	51	–	6–8
Autohemolysis			
After 24 hours			
Without glucose	1.3	1.5	0.05–0.5
Glucose added	1 1	1.5	0.0 –0.4
After 48 hours			
Without glucose	4.5	5.0	0.4 –4.5
Glucose added	1.2	7.0	0.05–0.4

He was re-examined in June 1961 There was no enlargement of the liver or the spleen. The most important laboratory data are listed in table 1 The bone marrow was hypercellular with a high percentage of normoblasts. The serum bilirubin concentration was 1 9 mg/100 ml, and the serum iron 224 μg/100 ml. Coombs test was negative.

Case 2. A seventy year-old female, she was the mother of case 1 She had been anemic and slightly jaundiced for many years. Laboratory findings Hemoglobin 7.2 g/100 ml, red cell count 2.5 millions, reticulocytes 5.2 per cent. Serum bilirubin concentration 2.8 mg/100 ml. The morphology of the red cells and the osmotic fragility were normal. By X-ray examination a gallstone was found.

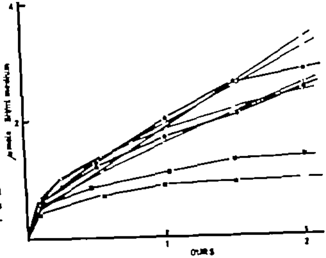


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showed an oxygen uptake within the normal range.

Similar results were obtained by the disulfide reduction experiments (fig. 2). A rapid initial appearance of cysteamine was observed, corresponding to a spontaneous exchange of cysteamine with intracellular glutathione. In the two cases of type I only a slight further reduction of cysteamine was observed, showing that the enzymatic reduction of disulfides was absent or nearly absent.

In case 1 and in three healthy individuals the red cell G-6-PD was measured directly. In the normals the enzyme activity was easily detected whereas the red cells from the patient showed no measurable activity. However, experiments with uniformly ^{14}C-labelled glucose and methylene blue revealed formation of a small amount of radioactive carbon dioxide, showing that the enzyme activity was not completely absent.

In case 3 (type II) the red cell content of AMP, ADP and ATP was within the normal range.

Discussion

Hockwald et al. (9) described in 1952 a new type of hereditary hemolytic anemia in American negroes. These patients got an acute intravascular hemolysis after primaquine treatment. Several drugs have since been found to provoke such hemolytic episodes in these patients (10). Fava beans can give a similar picture (23, 24). Beutler et al. (11, 12) found a decreased content of reduced glutathione (GSH) in the drug sensitive erythrocytes, and described the "*glutathione stability test*" which demonstrates that GSH of drug-sensitive erythrocytes disappears more rapidly in the presence of acetyl-phenylhydrazine than GSH of normal cells. Carson et al. (13) demonstrated a decreased G-6-PD activity in erythrocytes from primaquine-sensitive individuals, and later Ramot et al. (14) found a factor in the stroma of normal red cells, which activated G-6-PD in hemolysates of drug-sensitive red cells. A decreased G-6-PD activity is found in several cases of hereditary non-spherocytic hemolytic anemia (15, 16, 17). Further, a low red-cell GSH content and abnormal glutathione stability test is found in a few of these patients (17, 19). A decreased content of ATP is demonstrable in some patients suffering from this disease (18, 20).

Thus, the hereditary non-spherocytic hemolytic anemias and the drug-provoked hemolytic anemias seem to be related, both having a reduced G-6-PD activity and a decreased red-cell GSH content. Further, it seems probable that there are at least two groups of hereditary non-spherocytic hemolytic anemia based upon the enzyme studies, one group in which the G-6-PD activity is low and another in which G-6-PD activity is normal and the red-cell ATP content is reduced. It is not known whether these groups correspond to Dacie's type I and II.

On the basis of our studies we have concluded that hereditary non-spherocytic hemolytic anemia of type I is due to a deficiency of red-cell glucose-6-phosphate dehydrogenase activity. In type II we have not been able to detect any defects in carbohydrate metabolism. However, these findings are exactly converse to the pattern of the autohemolysis. In type I glucose inhibits the hemolysis and in type II there is increased autohemolysis on addition of glucose. Therefore, in type II we would expect greater abnormalities in the glucose metabolism than in type I. Perhaps the explanation

is that the hemolysis is considerably more rapid in the patient suffering from the type I as demonstrated by measurement of the life span of [51]Cr-labeled red cells. We cannot explain the hemolytic anemia or the abnormal autohemolysis of the patient suffering from the type II on the basis of our studies. The abnormal autohemolysis, which increases on addition of glucose, indicates here also there may be a defect in glucose metabolism.

In our studies we found in type I a decreased G-6-PD activity lowered oxygen uptake and decreased disulfide reduction in the red cells. In type II the autohemolysis shows greater abnormalities than in type I but in spite of this we have not been able to find any metabolic defect.

The oxygen uptake of red cells in the presence of methylene blue is almost completely due to an increase in the activity of the pentose shunt, the first enzyme of which is G-6-PD (21) Therefore the reduced oxygen uptake may be explained by the low G-6-PD-activity Cysteamine is reduced via exchange with glutathione, and the oxidized glutathione thus formed is reduced by TPNH and glutathione reductase (22) As the oxidative pentose shunt is the only source of TPNH in the red cells, cysteamine reduction depends on G-6-PD activity

By increasing the concentration of cysteamine, its own reduction is inhibited, even in normal erythrocytes. This inhibition is primarily due to an inhibition at the hexokinase level. Thus in disulfide-intoxicated cells, glycolysis is inhibited, and hence also the production of ATP (25) Probably oxidized glutathione (GSSG) also has such an inhibitory effect on hexokinase. In disulfide intoxicated cells, therefore the ATP level decreases rapidly even the presence of glucose. When nucleotides (adenosine or inosine) are added to such intoxicated cells, hexokinase is bypassed and the glycolytic production of ATP and the enzymatic reduction of disulfides again take place (25) Such a disulfide intoxication" may well be more easily created in erythrocytes which lack G-6-PD Primaquine has been shown to catalyze the oxidation of TPNH to TPN (26) and acetylphenylhydrazine has been shown to catalyze the oxidation of glutathione both in G-6-PD deficient and in normal cells (30) but in normal cells the oxidized glutathione formed is immediately reduced by glutathione reductase and TPNH In the G-6-PD deficient cells the production of TPNH is lacking or very low and no reduction of GSSG will take place. The GSSG will accumulate and probably inhibit the hexokinase of the cells.

It has been shown that erythrocytes lyse when the ATP content of the cells decreases below a certain level (26 27) and also that the lysis of G-6-PD deficient cells is preceded by a drop both in GSH (28) and in ATP (26)

Thus in erythrocytes lacking G-6-PD the following sequence of events probably leads to the hemolysis Oxidation of GSH t the disulfide GSSG—→inhibition at the hexokinase level—→inhibited glycolysis—→drop in ATP level—→lysis. *This mechanism is probably common to* Dacie type I patients and to the drug-sensitive group of Hockwald et al. (9) and the only difference may be that in Dacie's type I the G-6-PD activity is so low that this mechanism results in manifest disease even without drugs catalyzing the oxidation of GSH. Qualitatively Kirkman et al. (31) found some small biochemical differences on purifying the enzyme from congenital non-spherocytic

showed an oxygen uptake within the normal range.

Similar results were obtained by the disulfide reduction experiments (fig. 2). A rapid initial appearance of cysteamine was observed, corresponding to a spontaneous exchange of cysteamine with intracellular glutathione. In the two cases of type I only a slight further reduction of cysteamine was observed, showing that the enzymatic reduction of disulfides was absent or nearly absent.

In case 1 and in three healthy individuals the red cell G-6-PD was measured directly. In the normals the enzyme activity was easily detected whereas the red cells from the patient showed no measurable activity. However experiments with uniformly ^{14}C-labelled glucose and methylene blue revealed formation of a small amount of radioactive carbon dioxide, showing that the enzyme activity was not completely absent.

In case 3 (type II) the red cell content of AMP, ADP and ATP was within the normal range.

Discussion

Hockwald et al. (9) described in 1952 a new type of hereditary hemolytic anemia in American negroes. These patients got an acute intravascular hemolysis after primaquine treatment. Several drugs have since been found to provoke such hemolytic episodes in these patients (10). Fava beans can give a similar picture (23, 24). Beutler et al. (11, 12) found a decreased content of reduced glutathione (GSH) in the drug sensitive erythrocytes, and described the "glutathione stability test" which demonstrates that GSH of drug-sensitive erythrocytes disappears more rapidly in the presence of acetyl phenylhydrazine than GSH of normal cells. Carson et al. (13) demonstrated a decreased G-6-PD activity in erythrocytes from primaquine-sensitive individuals, and later Ramot et al. (14) found a factor in the stroma of normal red cells, which activated G-6-PD in hemolysates of drug-sensitive red cells. A decreased G-6-PD activity is found in several cases of hereditary non-spherocytic hemolytic anemia (15, 16, 17). Further a low red-cell GSH content and abnormal glutathione stability test is found in a few of these patients (17, 19). A decreased content of ATP is demonstrable in some patients suffering from this disease (18, 20).

Thus the hereditary non-spherocytic hemolytic anemias and the drug provoked hemolytic anemias seem to be related, both having a reduced G-6-PD activity and a decreased red-cell GSH content. Further it seems probable that there are at least two groups of hereditary non spherocytic hemolytic anemia based upon the enzyme studies, one group in which the G-6-PD activity is low and another in which G-6-PD activity is normal and the red-cell ATP content is reduced. It is not known whether these groups correspond to Dacie's type I and II.

On the basis of our studies we have concluded that hereditary non-spherocytic hemolytic anemia of type I is due to a deficiency of red-cell glucose 6-phosphate dehydrogenase activity. In type II we have not been able to detect any defects in carbohydrate metabolism. However, these findings are exactly converse to the pattern of the autohemolysis. In type I glucose inhibits the hemolysis and in type II there is increased autohemolysis on addition of glucose. Therefore, in type II we would expect greater abnormalities in the glucose metabolism than in type I. Perhaps the explanation

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anemia and comparing it with G-6-PD from a primaquine-sensitive and a normal person. But these differences probably have no influence on the mechanism of hemolysis.

In this connection it is interesting to note that also in at least some of Dacie's type II patients there seems to be a deficient ATP production (18, 20), the cause of which is not known.

It is then reasonable to suggest that in all cases of non-spherocytic hemolytic disease the common cause of hemolysis is a drop in the ATP level of the cells below a critical value.

Summary

The clinical picture in four patients with congenital non-spherocytic hemolytic anemia is described.

The first case had a moderate anemia but a pronounced hemoglobin breakdown. The mother demonstrated the same clinical and hematological picture, whereas twelve other members of the nearest family were healthy and showed no hematological abnormality. Glucose 6-phosphate dehydrogenase activity was absent, the erythrocytes showed a reduced oxygen uptake when stimulated with methylene blue, and the capacity to reduce the disulfide cysteamine was decreased. The hematological findings are in accordance with type I (Dacie).

The third case had anemia and jaundice since early childhood. At the age of 14 an enlarged spleen was removed without influence on the clinical picture. The hemoglobin breakdown was pronounced, and, as in the other patient, there was a shortened life-span of the erythrocytes. The mother showed a moderate anemia and an abnormal autohemolysis. The hematological findings in son and mother were in accordance with type II (Dacie). The biochemical investigations revealed with our methods a normal glycolysis and AMP, ADP and ATP in the red cells were within the normal range.

The mechanism of hemolysis in the different types of hereditary non-spherocytic hemolytic anemia has been discussed and it is suggested that the common cause of hemolysis in these diseases is a drop in the ATP-content of the erythrocytes below a critical level.

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From the University Medical Clinic (Renal Clinic) Lund, Sweden

Oral Aluminium Hydroxide in the Treatment of Hyperphosphatemia and Acidosis in Acute and Chronic Renal Insufficiency

By

Tore Lindholm

Salts of aluminium taken orally are almost completely recovered in the feces (McKenne 1930, 1931) Soluble salts of aluminium form with phosphate insoluble salts which pass through the intestinal canal without being reabsorbed. Oral therapy can therefore lead to a marked lowering of the serum-phosphate concentration (Cox et al. 1931) This effect has been utilized therapeutically both to prevent or control retention of phosphate in renal failure (Freeman and Freeman 1941) and to prevent the formation of phosphate-containing urinary calculi (Shorr and Carter 1950)

At this clinic treatment with aluminium hydroxide in renal insufficiency has been used for more than 15 years (Alwall 1954) Such treatment is also recommended by Merrill (1955) Bland (1956) and Reubi (1960)

Up to 1957 the only form in which aluminium hydroxide was available for our therapy was as tablets. Several hundreds of patients with hyperphosphatemia and acidosis associated with acute and chronic renal failure were treated and good therapeutic results were obtained in a great number of cases. But many uremic patients did not tolerate the large doses required, 10 to 30 tablets per day The lower dosage recommended by Reubi (1960) can be insufficient for the attainment of a therapeutic effect.

After discussion of these difficulties AB Hässle, in 1957 placed at our disposal an Alucol' suspension with the following formula

Aluminium hydroxide	8.5—9
Glycerin	5 %
Preserving medium	
Flavouring	q. s.
Water	ad 100 %

This suspension was used for 2 years. As from August 19.9 we have used 'Novalucol' suspension (Hässle) with the following formula

Aluminium hydroxide	6 g
Magnesium hydroxide	2.5 g
Glycerin	10 g
Sorbitol	10 g per 100 g suspension.

The antacid effect of the suspension is better than that of a corresponding

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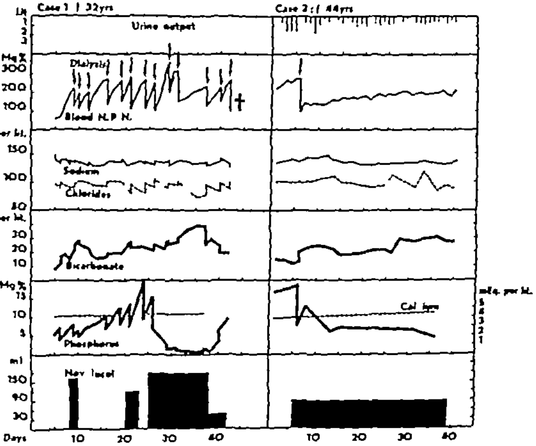


Fig 1

amount of tablets (Tomenius and Williams 1960) Its content of sodium is only 0 1222 g per 100 g suspension, which seems to be of no practical significance in this series of patients.

Material and results

At this clinic treatment with the artificial kidney was started in 1946 and the clinic admits patients *with severe and* complicated renal diseases from a large area of the country Every year about 100 cases of anuria-oliguria are treated with the artificial kidney These are in the main, patients with acute renal disease most of them with acute renal failure as a complication of some severe surgical disease. Operations, infections and fever etc. often cause a rapid break down of protein with resultant hyperphosphatemia and acidosis. Anuria-oliguria is attended with the risk of electrolyte and fluid retention. Therefore, it is often potentially dangerous to try to neutralize the acidosis by administration of, for instance sodium bicarbonate as a solution or as a dry powder Such measures may lead to electrolyte-fluid retention and pulmonary and/or cerebral edema.

Administration of 1—2 tablespoonfuls of Novalucol suspension three times per *day (45—90 g) is in many cases* sufficient to control hyperphosphatemia and acidosis. If because of vomiting or coma the patient is unable to drink it, the solution is given *through a gastric* tube.

With this routine procedure, dialysis on the indication of hyperphosphatemia and acidosis is, as a rule, not necessary.

The following case-records illustrate the effect of the treatment (fig. 1).

Case 1. A 32-year-old woman was admitted elsewhere as case of criminal septic abortion. Despite evacuation of the uterus and intensive antibiotic therapy her condition deteriorated. Anuria developed after prolonged severe shock, which was controlled with large amounts of fluid, noradrenaline, etc. The patient was transferred to this clinic and had thirteen treatments with the artificial kidney. On account of bronchial complications tracheotomy was performed, respiratory insufficiency necessitated treatment in respirator. Her general condition was throughout poor. She died after 6 weeks. Autopsy showed cortical necrosis in both kidneys, among other findings.

Infection and a high rate of protein breakdown led to hyperphosphatemia and acidosis, which were, with difficulty corrected by the dialysis treatments. The effect of 'Novalucol' suspension by mouth promptly solved this part of the therapeutic problem, and moreover with the dosage used, the drug produced hypophosphatemia and alkalosis. When the dose was reduced, hyperphosphatemia developed again.

The following case-record shows the effect of 'Novalucol' suspension on the serum-phosphate concentration in a case of chronic pyelonephritis with nephrocirrhosis.

Case 2. A 44-year-old woman had had established proteinuria for the last 22 years and slight uremia for the last 8 years. After few months of increasing fatigue, nausea and vomiting she was admitted to this clinic in poor general condition. N. P. N. exceeded 200 mg per 100 ml and she had fluid retention (edema of the face, "fluid lung" etc.). To correct the uremic intoxication, acidosis and fluid retention, she was treated with the artificial kidney (dialysis and ultrafiltration). By continued administration of 'Novalucol' suspension the tendency to phosphate retention was offset. Since the patient was polyuric her condition improved on high fluid-intake.

A great number of patients with chronic renal insufficiency with tendency to hyperphosphatemia and acidosis, resulting chiefly from chronic pyelonephritis with nephrocirrhosis, have received longterm continuous ambulatory treatment with 'Novalucol' suspension, when the therapeutic problem could not be solved by giving sodium-bicarbonate, etc. During treatment with aluminium hydroxide the intake of protein can often be increased without causing a rise of serum-phosphorus. The nutritional status can thus be improved. The often troublesome uremic gastritis can many times be favourably influenced by 'Novalucol' suspension. Whether it is possible thereby to lessen the risk of mucous membrane bleeding in grave uremia, is still however an open question. Our experience leads us to believe that this can be the case.

Summary

Oral administration of aluminium hydroxide has for more than 15 years been used at this clinic as a means of reducing hyperphosphatemia and acidosis in the treatment of the large numbers of patients with acute and chronic renal insufficiency. Many of the uremic patients do not tolerate the large amounts of tablets necessary for obtaining the desired therapeutic effect. In the form of a suspension (for instance 'Novalucol suspension, Hässle) 3 to 6 g of aluminium

hydroxide can generally be given daily without difficulty This dosage is sufficient to restore serum-phosphorus to normal and may even produce a hypophosphatemia. The effect of such treatment which has been applied in about 300 cases over the last 2 years, is illustrated by two typical case-records. The procedure solves an important therapeutic problem *As a rule, it eliminates the need for administration of* for instance, sodium bicarbonate solution to neutralize an acidosis a potentially dangerous measure, particularly in oliguria anuria, where it may lead to electrolyte and fluid retention and cerebral and/or pulmonary edema In most cases treatment with the artificial kidney for acidosis will not be necessary

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The Department of Medicine, University of Manchester England

The Natural History of the Nephrotic Syndrome and the Assessment of the Effects of Treatment with Steroids

By

M. H. Roscoe

The patients studied were considered to have the nephrotic syndrome on the basis of marked proteinuria, hypoproteinaemia, hypercholesterolaemia and, in the majority oedema.

Fifty cases are reviewed, who have either been followed for 4 years or more or who died within this time, 38 of these were previously reported on (20) but survivors have now been followed for a further 6 years. Five other cases with a history of less than 4 years are dealt with briefly

The chief object of this study of the course of the disease is to provide a basis for therapeutic trials, but it can be said that since by now considerable number of the cases have been given steroids, the series no longer shows what happens when no treatment is given. It will however be shown that the course of the disease in patients having steroids is broadly similar to that in patients not on steroids, so that qualitatively there is no alteration. On the other hand there are quantitative changes, with an increasing rate of improvement in the cases who respond and probably an increase in the number of cases improving and remitting although the series is not large enough to prove the latter point.

Laboratory methods

Estimation of plasma proteins and blood urea were made in the Clinical Laboratory of the Manchester Royal Infirmary the former by the method of Reinhold (18) and the latter by the urease nesslerization method. Urinary protein was measured by turbidity produced with salicylsulphonic acid and is expressed as g. per lg. of creatinine (g/g Cr.) to allow for variations in urine concentration, the values so obtained approximating to the 24 hour excretion. Creatinine is measured by the Jaffe reaction, that of serum after removal of extraneous chromogens with Fuller's earth (22)

I The course of the disease in 50 cases of the nephrotic syndrom

Fig 1 gives the numbers and percentages of cases dying, lost sight of or not yet followed in each year of illness. It

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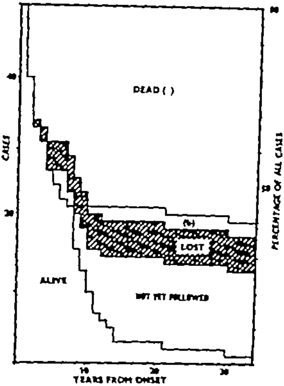


Fig 1 Death and survival of 50 cases of the nephrotic syndrome Dead a) from causes related to the syndrome b) from unrelated causes.

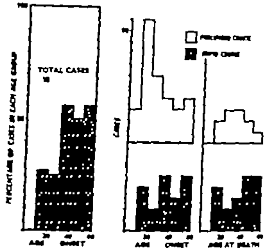


Fig 2. The age at onset of illness or death of cases with rapidly progressive disease or disease with a prolonged course

shows clearly that the mortality in the early years is high, the percentage of cases at risk dying being 20 in the first year 15 in the second 3 in the third and 6 in the fourth and 38 % of all cases being dead after 4 years. In the next 3 years there are no deaths, but in the eighth to the eleventh 9 so by the end of this year 56 % of all cases are known to have died as a result of the disease. Two others died within this time from unrelated causes.

The cases have been divided into two groups, those deteriorating rapidly and dying within 4 years (19) and those with a prolonged course (31)

The age at onset ranged from 7—57 years. Fig 2 shows, on the left, the proportion of cases starting in each decade, with rapid deterioration or with a prolonged course. Half the cases starting over age of 30 deteriorated rapidly but a smaller proportion of those starting under 30 The age of onset and numbers of cases with a rapid or prolonged course are shown in the centre. Rapid course cases start equally at all ages, but the majority of cases with a long course start early The numbers starting at less than 10 years are not meaningful, as children are not usually admitted here. In spite of the high incidence of the prolonged course in young people, deaths in this group did not occur predominantly at an early age (right hand side of figures) This is because the first signs often antedated the terminal illness by many years.

Table I summarises some features of the 50 rapid and prolonged course cases, which will now be considered separately

Cases deteriorating rapidly (19)

All these patients had oedema, severe in 16 and generally persisting till death. Two died without renal failure and one with only mild failure from causes related to the nephrotic state. The others all had nitrogen retention before death usually

Table I. Summary of 50 cases with the nephrotic syndrome

Oedema	Proteinuria	Yrs from onset: Followed without deterioration	Yrs from onset: Death from renal failure
Rapid course (19)			
Present throughout (14) / Lost terminally (2) / Not known (3)	Present throughout	2 died in 1st yr without renal failure	1 (8) / 2 (6) / 3 (1) / 4 (2)
Remission or prolonged course (31)			
None (3)	Persistent	4 10	8
Present (28)			
One attack only (10)	Final 0 (6)	1½ 2½ 3,3,4 7	—
	tr (3)	5,7 12	—
	+ (1)	5	—
Recurrent and persistent (18)	Final 0 (2)	9 13	—
	tr (2)	7 11	—
	+ (14)	7 7 13½ 20	7 7 8,6,9 9 10,10,20,29

No. of cases in parenthesis.

with severe hypertension and often retinopathy. Evidence of deterioration was present at an early stage. Six patients were seen within month of onset, 4 already having hypertension and nitrogen retention, one hypertension only and one neither. Nitrogen retention developed in all within the year. Six patients were seen 2—12 months from onset, only one being normotensive and he became hypertensive in a few weeks. All had hypertension by the end of the year. Five patients were not seen till they had had signs for 1—3 years, when they all had hypertension, nitrogen retention, or both. Thus there were only 4 out of 19 who had both normal blood pressure and normal blood urea, when first seen, and those who survived a year all had adverse signs by then.

Repeat creatinine clearances were not carried out on any of these patients, but the rapid deterioration in kidney function is illustrated by the fact that case 51 had a clearance of 76 ml/min. 1½ months from the onset and 8 months before death, and case 52 one of 85 ml/min. 1 month from the onset and 9 months from death.

One patient in this group is known to have had renal vein thrombosis and in two others it was suspected, but the majority of patients were seen before this condition was appreciated.

Cases with early remission or prolonged course (31)

In three oedema has never been present but they have been included because the proteinuria and hypercholesterolaemia, which they had, are often the precursors of the full syndrome. This was the case in three other subjects in this series, who developed oedema 3, 4 and 25 years after the finding of proteinuria.

Table II Ten cases with recurrent oedema followed throughout

Case no.	Sex	Age at onset (yrs)	Fol-low-up (yrs)	Oedema	Fin-ally free (yrs)	Proteinuria	Final		B. urea mg/100 ml	Ster oid dos-age
							Pro-tein-uria g/g Cr	B. P mm Hg		
15	F	19	13	Severe and per sistent for 3 yrs, gradually decreas-ing thereafter	9	Persistent for 8 yrs, decreas-ing	0	115/80	—	0
8	F	15	9	3 attacks in 1 t ? yrs, free 1 yr intermittent for next 1 / yrs	4 /	None between attacks	0	120/80	34	S + L
9	M	15	11	Intermittent for 7 yrs	4 /	None between early attacks	tr	110/60	22	S + L
41	M	12	7	3 attacks in 2 yrs	5	Reduction be tween attacks	tr	120/75	—	S
*0	M	25	33 (10)	Severe attack at onset free for 23 yrs, intermit tent for next 4 yrs	5	Not known in early yrs, pres-ent from 25th yr onwards, decreasing	4.0	170/95	67	0
6	M	14	13	Severe att cks 1st 3rd and 7th yrs, then intermittent for 2 rs	4	Always present	5.2	130/95	49	S + L
43	F	35	7	Intermittent for 5 yrs	2	Always present	+	170/90	30	S
47	M	35	7	Attacks 1 t 3rd and 7th yrs	—	None between 1st and 2nd attacks	+	120/90	36	S
16	F	*0	10 (died)	Slight 3 yrs after discovery of pro-teinuria. Severe during pregnancy 2 yrs later Inter mittent thereafter	0	Always present	+ +	160/110	64	S
17	F	21	7 (died)	Attacks 1st yr and 2nd yr when preg-nant. Mild and in-termittent 4th—7th yrs	0	Always present	+ +	190/155	310	0

S. 10—14 days treatment, see table VI L. Long term treatment see table VII

Not terminal, 7th yr

The 10 cases with only one attack of oedema include 4 who were not followed up after 3 years or less, and may possibly have relapsed, but the others have been followed for 4—12 years.

In many cases the patients were only seen at a late in their illness and early information is lacking. But in table II are shown some details of the complicated course of 10 who have been followed here for the greater part of their illness. In all there was heavy proteinuria during the attacks of oedema, but excretion generally decreased between attacks, being absent in three. No signs of renal failure are present in 7 of the 8 surviving cases the eight, No. 20 has a blood urea of 67 mg/100 ml, but it has been at this level for the past 6 years. Marked uraemia was present in the ones followed to death. The cases in whom proteinuria was lost or much reduced between attacks of oedema did well and have no residual signs, except in some a trace of proteinuria. Case 15 who had persistent proteinuria for 8 years, has also made a complete recovery but the other 5 cases with persistent marked proteinuria all have, or had, hypertension. In case 6 this developed in the second year when he was 16, but it has got no worse in the subsequent 11 years and in case 20 who was 48 when we first saw him, there has not been a sustained increase in 10 years. In the other 3 however the hypertension progressed. Case 43 had a blood pressure of 140/90 mm Hg when she was first seen in the third year and 190/120 mm Hg in the sixth year but this has since been reduced to 170/90 mm Hg by hypotensive drugs. The blood pressures and blood urea of case 17 who was followed by us from start to finish, are shown in fig. 3 Protein excretion was always present and in the last 3 years

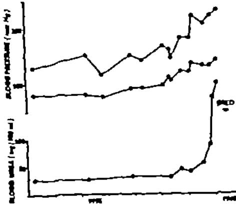


Fig. 3 Blood pressures and blood urea during 7 years in case 17

varied from 10—15 g/g Cr Three and a half years from the onset there was a slight increase in the diastolic pressure, but after 5 years a rapid increase. In the last year hypotensive treatment was instituted but with little success. The blood urea did not rise significantly till the sixth year when hypertension was marked. Death from congestive cardiac failure and renal failure occurred after 7 years.

This case would seem to be typical of a number of those with a prolonged course deterioration being heralded by the onset of hypertension and renal failure appearing later Table III shows the 11 cases who have died. In 8 severe hypertension had been present for a year or more before death and before any considerable elevation of the blood urea, and repeat measurements showed increasing hypertension, often with only slow or slight increases in blood urea. In contrast to the cases with an acute course, terminal oedema in these cases was generally not severe and often largely due to cardiac failure.

Creatinine clearances have not been obtained routinely on these patients, who were most usually followed as out-patients

Table III Blood pressure and blood urea in patients with a long history of the nephrotic syndrome who died

Case no.	Yrs from onset	B. P mm Hg	Urea mg/100 ml	Yrs from onset	B. P mm Hg	B. urea mg/100 ml	Yrs from onset (died)
1	8	135/85	78	—	—	—	9
2	5	190/110	28	6	180/1?0	52	10
3	8	135/90	44	8½	170/100	54	9
10	7	220/125	44	8	190/140	50	8
11	26	165/95	—	29	165/95	108	29
16	5	170/100	44	7	160/110	45	10
17	5	180/120	4	6	185/130	59	7
19	6	240/125	32	7	240/130	40	8
23	5	150/110	22	6	210/130	32	7
35	3	180/120	66	6	10/140	76	8
50	19	145/85	56	19½	165/115	10?	20

Table II Blood pressure and blood urea during attacks of oedema and after

Case no.	Blood pressure mm Hg		Blood urea mg/100 ml	
	During	After	During	After
8	140/100	120/80	58	30
9	140/90	130/80	60	20
47	130/95	120/80	78	36
48	130/95	120/85	54	44

but case 9 6 years from the onset, after his last attack had a clearance of 156 ml/min and case 6 after persistent proteinuria for 13 years a clearance of 118 ml/min

Hypertension, nitrogen retention and creatinine clearance during attacks of oedema

Patients who deteriorate rapidly are distinguished by the early appearance of hypertension and/or nitrogen retention and these signs are present also in subjects with a prolonged course when entering the final stage of deterioration.

However essential hypertension may be present and misleading for instance case 32 had a blood pressure of 145/110 mm Hg at the outset but no deterioration in the next 12 years. A mild degree of both hypertension and nitrogen retention may also be seen in some patients with severe oedema these being lost when the oedema goes. This is shown for 4 subjects in table IV All made good recoveries, two now having no proteinuria, two only a trace. Steroids had not been given when the abnormal values were found.

There is also in some cases a marked reduction in creatinine clearance during attacks of oedema. To some extent this may be related to the low urine volumes at this time, which even in normal subjects may be accompanied by a lowered filtration rate (19 21) but a true depression is also present. This is shown for 2 cases in table V The clearance increased by 50 % after diuresis in case 55 in case 58 it quadrupled The rise was not associated with any significant alteration in plasma urea in case 55 this being normal throughout, but there was a fall from an abnormal value to a normal one in case 58.

In these 2 cases diuresis was produced by ACTH but a rise in clearance may

Table V: Creatinine clearances in patients when oedematous and after diuresis. 24 hour urine specimens

Case no.	Period and no. of days	Urine vol. ml/min		Creatinine				Plasma urea mg/100 ml
				Serum mg/l	Excretion µg/min	Clearance		
		Range	Mean			ml/min	/1.73 sq.m	
53	Pre-diuresis (13)	0.505–0.825	0.693	12.1	740	61	62	26, 31
	Diuresis (10)	1.505–2.84	1.89	8.0	785	98	100	18, 28
	Post-diuresis (4)	0.555–1.50	0.915	6.9	605	90	83	24
56	Pre-diuresis (15)	0.09 –0.295	0.225	13.75	223	16	32	56
	Post-diuresis (5)	0.365–0.99	0.665	5.9	260	44	83	32
	Post-diuresis (9)	0.385–0.79	0.53	4.6	320	69	134	21

also occur when no steroids are given. Thus in case 8 there was an increase of 50 after a slow diuresis with resin and mercurial diuretics.

Case 20 has had startling changes. He had a history of a severe attack of oedema 25 years before we saw him and was admitted at the age of 50 with marked oedema, blood pressure 180/95 mm Hg falling to 165/80 mm Hg and blood urea from 56 to 30 mg/100 ml. The oedema persisted with slight remissions and when he was readmitted 5½ years later the blood pressure was 220/130 mm Hg, blood urea 238 mg/100 ml. He did not respond to diuretics, but after removal of much fluid with Southey's tubes the remaining oedema gradually went and 6 months later he had none, but a blood pressure of 160/90 mm Hg and blood urea of 260 mg/100 ml. Six months later to everyone's intense surprise, he was alive and the blood urea was 58 mg/100 ml. In the 6 years since then he has had no oedema, decreasing proteinuria, a blood pressure in the neighbourhood of 170/90 mm Hg and blood ureas of from 36 to 70 mg/100 ml. He is however disabled by general weakness and severe gastric pain and flatulence, which have been his major symptoms throughout the illness and for which no explanation has been found. At no time has he had steroids.

2. The effect of steroids

Only 2 of the cases with the rapidly progressive condition were given steroids (Nos. 52 and 53, table VI). A diuresis was not produced in either case and no improvement was seen.

Twelve of the 31 cases, remitting or with a prolonged course, had steroids. Six only had short courses, 4 had short and long courses and 2 long courses only. Although it is thought that the course of the disease was altered in some patients by this therapy, the picture produced is not different from that seen in other patients who had no steroids. It will be shown that the rapid diuresis and restoration of normal serum and urine protein levels occurs both with and without treatment and table II illustrates the fact that improvement and remission occur after years of the disease, in both treated and untreated cases: case 8 had steroids from the fourth year and became free of oedema and proteinuria, but case 15, who never had steroids, has also been free of all signs for 4 years after 8 years of proteinuria.

Table VI The diuretic effect of steroids

Case no.	Age (yrs)	Time from onset (yrs)	Steroid given (10—14 days)	Diuresis: During dosage	Diuresis: On withdrawal	Diuresis: None	Proteinuria g/g Cr: Pre	Proteinuria g/g Cr: Post.	Proteinuria g/g Cr: Wks	P albumen g/100 ml: Pre	P albumen g/100 ml: Post.	P albumen g/100 ml: Wks	Subsequent course
6	15	6	A	+	—	—	14 0	6.0	(½/)	1.5	3.0	(3)	Relapsed
	16	7	A	+	—	—	23.1	7 4	(2 /)	1 4	2.1	(3)	Relapsed
	16½	7 /	P	—	+	—	1 9	15.0	(2 /)	1.2	1.9	(2)	Improvement with L T
8	16½/	1 /	A	+	—	—	14.3	0.2	(2)	1 1	3.0	(3)	Improvement with L T
9	17	5½/	[illegible]	+	—	—	19 0	0.5	(1)	1.9	3.4	(4)	Relapsed
	18½/	5	A	+	—	—	15.1	0.2	(1 /)	1.2	3.5	(3)	Relapsed
	19½/	6	A	+	—	—	0.0	0.8	(1 /)	1.5	4.5	(7)	Relapsed
	20	6½/	P	+	—	—	+	tr	(2)	1.8	2.9	(3)	Improvement with L T
41	12	2	[illegible]	+	—	—	++	0	(2)	1 4	2.7	(2 /)	Steady improvement
57	17	/	P	+	—	—	21 0	1 0	(2)	1 4	2.6	(2)	Improvement with L T but relapsed
		/	A	+	—	—	14 0	0	( )	1.5	3.7	(3)	Improvement with L T
48	51	/	P	+	—	—	5.2	5.9	(2 /)	1.8	1 7	(4)	No improvement with L T
47	35	0	0	—	—	—	++	tr	(4)	1.2	3.1	(3½/)	Spontaneous remission
	37	2	P	—	+	—	10.0	10.9	(3)	1.2	0.8	(4)	Remission with resin
	41	6	0	—	—	—	10.3	6.9	(9)	1 7	3.1	(9)	Remission with resin
43	33	1 /	A	—	+	—	20.0	12.0	(2)	1.6	2.3	(7)	Relapsed
55	21	2 /	A	—	+	—	12.7	9.9	(2)	1 4	1.3	(3)	Relapsed
41	47	/	A	—	—	0	5.7	5.5	( )	1 0	1 4	(3)	Not followed
45	47	/	A	—	—	0	8.4	7 4	(3)	1 1	1 0	(3)	Improvement with mercurials
54	22	1	P	—	—	0	6.3	4 0	(1 /)	1 7	2.1	(3 /)	Improvement with resin
16	28	8	A	—	—	0	9.1	9.9	(3)	1.8	2.6	(3)	Deteriorated
23	37	7	P	—	—	0	8.4	8.8	(2)	2.3	2.5	(3½/)	Renal failure present and progressed
50	45	19	P	—	—	0	15.0	—	—	1 7	1 4	(3)	
32	34	/	P	—	—	0	10.5	15.7	(2)	0.6	1 0	(2)	
53	47	1	C	—	—	0	18.9	20.0	(3)	1.8	1.5	(7)	

A = ACTH 20 units b. d. P = Prednisone or prednisolone 10 mg b. d.
C = Cortisone 300 mg daily L. T = Long term prednisone dosage

Thus the proportion of patients remitting or remaining well in spite of continuing residual signs, may be altered by therapy but the inclusion of treated patients in the series does not introduce any phenomenon which cannot be duplicated in untreated patients.

The two aspects of steroid treatment which have been investigated are its diuretic effect and the influence of long term therapy in reducing proteinuria, increasing serum albumen concentrations and preventing relapses.

Steroids as diuretics

Table VI shows the result of giving steroids to 17 patients when they were oedematous, 3 on several occasions. In 6 diuresis started during dosage and in 5 of these there was a rapid decrease in

Table VII. Long term prednisone treatment. The stated dose was given on 3 consecutive days in each week

Case no.	Age (yrs)	Time from onset (yrs)	Previous ACTH (A) or prednisone (P) and diuretic response	Prednisone, mg/day	Condition at start and after ( ) weeks of treatment: Oedema	Proteinuria, g/g Cr	Plasma albumen, g/100 ml	Wks	Later treatment: Prednisone	Other	Final condition: Proteinuria, g/g Cr	Plasma albumen, g/100 ml	Yrs from start of treatment
4	16½	7/12	A + P (Good response)	20	0	15—9	1.9—3.4	(17)	Continuous for 4 yrs (then see fig. 4)	None	4	4.1	4
8	19½	3/12	A (Good response)	20	+	10—tr	1.9—4.3	(5)	Continuous for 4 yrs	None	0	4.7	4½
9	20	5/12	A + P (Good response)	5	0	tr.—tr	2.9—4.6	(7)	10 mg for 4½ yrs	Remn	tr.	4.4	4½
57	17	9	P (Good response)	20	0	11—tr	2.1—4.3	(20)	10 mg. Relapsed 30 mg	Remn	0	4.3	1
46	51	4/12	P Diuresis but no improvement in chemistry	20	+	6—14	1.7—1.7	(6)	—	—	—	—	—
				40	+	—6	—2.0	(7)	Reduced and stopped 4/12 later	Remn	1	4.5	5
54	22	1	P No response	20	+	4.5—4	2.0—1.8	(11)	—	Remn and chlorothiazide	2.5	2.9	2½
46	8	4/12	None	10	0	4—0	1.4—4.5	(46)	5 mg for /12	None	0	4.3	2½
56	38	/12	None	20	0	5—6	2.4—2.2	(22)	Reduced and stopped /12 later	Remn	0.5	4.8	2
49	16	2½/12	None	20	None ever	6—4	3.3—2.8	(30)	—	None	9	2.7	1

proteinuria and increase in serum albumen. Three other cases lost their oedema as soon as the steroids were stopped but decrease in proteinuria and increase in serum albumen were slight or absent. In 8 patients no diuresis occurred and there was little or no alteration in chemistry

All the patients who had a rapid response and improvement in chemistry were young 12—20 years old at the time. None of the older patients responded so well and two of 21 and 22 years also showed little benefit The duration of the disease does not seem to influence the effectiveness of treatment, cases 6 and 9 responding well in the 7th year of illness. When nitrogen retention was present however diuresis was not observed

The cases responding well had a more rapid diuresis decrease in proteinuria and rise in serum albumen than we have seen with any other form of therapy but similar changes have been seen to occur spontaneously Thus case 6 in the second year of his illness, had a massive diuresis when no specific treatment was being employed but proteinuria was not lost and increase in serum albumen was slow Case 47 was probably unaffected by the prednisone he had in his second attack of oedema, diuresis coinciding with withdrawal and the giving of mercurial diuretics and little change in proteinuria occurring On the other hand in his first attack 2 years before he had a rapid diuresis with no treatment except a low salt diet, and a decrease in proteinuria and rise in serum albumen as good as any seen with steroids. In a third attack 4 years later diuresis again occurred without any steroid treatment but the serum albumen increased more slowly than before and proteinuria was still marked after 4 months

The only complication seen with the dosage used 20 units twice daily of ACTH or 10 mg twice daily of prednisone for 10 to 14 days was one case of acute psychosis. No attempt was made to see whether those who did not respond would do so with larger doses The only case with a high dosage, No. 53 with 300 mg daily of cortisone for 3 weeks, had a raised blood urea and treatment was given as a last resort, although he was known to have a history of peptic ulcer There was no response and he died 3 weeks later from a gastric haemorrhage.

A good outcome of this short term therapy is not necessarily followed by continued improvement. Case 41 with a rapid diuresis, had no further treatment and now $4^1/_2$ years later has only minimal proteinuria, but the others who responded rapidly relapsed unless they were given long term treatment.

Long term treatment with prednisone

These results are shown in table VII. The four cases (6 8 9 and 57) who had had a good diuresis and increase in plasma albumen with ACTH or prednisone improved markedly Case 9 had two mild relapses in the first year when only having 5 mg and case 57 relapsed when the dose was reduced from 20 to 10 mg but is now free of proteinuria on 30 mg The two cases (48 and 54) in whom no improvement in plasma albumen had occurred when prednisone was given to promote diuresis, showed little or no change. The dose was doubled in case 48 but without effect. One of the previously untreated cases (46) had a good response but the other two (56 and 49) did not.

Of the 4 cases showing no benefit from treatment Nos. 48 and 56 have improved slowly but steadily while receiving resin only and now 5 and $2^1/$ years from the start of their illness have only slight proteinuria and normal or near normal plasma albumens. Case 54 has also improved slowly and although the plasma albumen is not yet normal she no longer has oedema and is in regular employment. She is the only case followed for any length



Fig. 4 Variations in plasma albumen and urinary protein in case 6 followed for 13 years from the onset of the illness.
A = ACTH C = cortisone; P = prednisone, the doses shown being given daily 3 consecutive days in each week. Oedema was generally present when the plasma albumen level was less than 2.5 g/100 ml.

of time who has had a renal biopsy and this, in the second year of her illness, showed typical membranous glomerulonephritis (Dr G M. Berlyne) Case 49 who has never had oedema, has increasing proteinuria and a falling plasma albumen.

Since spontaneous recovery may occur without steroid therapy it is always possible that observed improvement may have been coincidental. This is possible in case 46 where improvement was slow and in case 57 but the previous histories of cases 6, 8 and 9 suggest that the long term prednisone treatment was responsible for their improvement. The histories of these 3 patients are given in the previous paper (20) up till the autumn of 1955 They all had either repeated attacks of oedema or persistent oedema, which was removed in the later years by giving ACTH, but recurred even when long term cortisone was given. From the time that prednisone was given in adequate amounts they had no oedema and the serum albumen was maintained at normal levels. It was continued in all 3 for 4 years and then tailed off and stopped. Case 8 with no proteinuria and case 9 with only a trace have not deteriorated in the 14 and 12 months respectively since treatment stopped, but case 6, who never lost his proteinuria showed an increase in this and decrease in serum albumen when he had had no treatment for 6 months. Treatment was restarted and the serum albumen returned to normal but 4 months later he failed to take his prednisone for 4 weeks and developed frank oedema recovering when treatment restarted The serum albumen and proteinuria during the 12 years this patient has been followed are shown in fig 4

Discussion

The course of the nephrotic syndrome as illustrated by these cases, is very variable. At the one extreme there are those who have a single attack of oedema, proteinuria and hypoalbuminaemia from which they recover and after which they have no further trouble, and at the other extreme are those who do not recover from their first attack, who show evidence of renal failure or hypertension within the first year and die, often in this year or within a few years. Between these extremes are found patients who do not recover from the first attack or who apparently recover and then relapse and in whom abnormal signs, sometimes oedema, are present intermittently or per

sistently for many years. Some of these, even after several years, lose all evidence of disease though only life time studies can show whether such remissions are permanent. Others after varying times during which they may have had periods without symptoms, are seen with terminal renal failure usually preceded by severe hypertension. Oedema in these final attacks is generally less marked than in earlier ones and to some extent due to congestive heart failure, this being a point of difference from the persistent severe oedema seen terminally in most cases with a rapid course.

A very similar picture is given by Johnson and Reader (9) who followed 80 cases of all ages without known complicating systemic disease. Thirteen cases died within two years, often with persistent gross oedema and elevated blood pressure and blood urea from the first attack. Twenty four cases were alive and followed for 2 to 20 years from the first attack, 8 being in complete remission. Two thirds of their cases had raised blood pressure or blood urea or haematuria at the start but this high incidence may be due to failure to distinguish temporary abnormalities which can occur during acute oedematous episodes. In the present series a third of the patients had early hypertension or renal failure leading to death within four years, but this is probably an overestimate of the incidence of acute course cases, owing to selective hospital admission.

A number of papers have recently been published in which classification of cases of the nephrotic syndrome is made from the pathology of kidney biopsy specimens (8, 10, 25, 27). The main division is, into those cases in which the kidney lesion is part of a systemic disease such as amyloidosis, thrombosis involving the renal vein, systemic lupus erythematosus or diabetes, and the rest without known complicating conditions. In the first group the prognosis is bad. Without pathological evidence it is not possible to known whether systemic disease was present in our cases, but it is probable that it was in some of those dying in the early years, especially as the majority of these were seen before the relation of these conditions to the nephrotic syndrome was realised.

The persistence of the condition in a benign form is probably not sufficiently realised, for which two gloomy concepts of the disease are largely responsible. Ellis (3) states that, unless rapid recovery occurs (5 cases out of 145 in his series) 'there is only one course with steadily progressive renal destruction'. But 11 of his 38 cases dying of uraemia lived for 5 to 10 years, one longer and 6 cases were alive after 10 years, 4 after 20. Addis (1) considered that in the majority of cases the nephrotic syndrome is the 'degenerative' stage in nephritis, preceded by an attack of acute nephritis and followed by the final stage of N retention, and characterised throughout by a progressive loss of nephrons.

More recent work, however, gives no evidence that there is any loss of nephrons or irreversible renal damage in many cases with the nephrotic syndrome. Increased blood ureas and decreased filtration rates, in the acute oedematous stage, have been shown by us and by others (5, 7, 13, 15) to return to normal when diuresis has taken place, and the most characteristic sign of abnormal renal function, the high protein excretion, may be recovered from. Even when proteinuria persists it is doubtful whether this indicates continuous disease, Vernier (26) maintaining that it does, but Lange

(11) denying it. Biopsies have not been practised for long enough to give the answer. Progression of lesions in cases doing badly have been recorded (8, 28) and regression when proteinuria was reduced or lost (4, 8, 14) but after too short an interval for the ultimate course of the disease to be known.

The fact remains, however, that the cases who have died, often after many years of apparent quiescence, had renal failure. This, in the majority of our cases, was preceded by the development of hypertension, which was often present for years before the blood urea rose (fig. 5, table III), a finding which suggests that whatever causes persistent proteinuria also induces hypertension and that this leads to nephron degeneration and nitrogen retention, rather than the reverse process of nephron degeneration inducing hypertension. There is no very definite relationship between the degree and duration of proteinuria and the onset of hypertension, and retrospective investigation of this is difficult because intermittent proteinuria is known to occur (25) and adult patients with proteinuria and a history of oedema in early life, for example cases 20 and 50 in this series, may well have been free in the intervening years. But in our cases studied from the start, hypertension developed in those with the most severe and unremitting proteinuria, while those who lost their proteinuria between attacks, although it was massive during them, remain well as yet.

Treatment should therefore be aimed at removing the oedema, which is the condition of most immediate concern to the patient, at reducing the proteinuria and at lowering the blood pressure, as soon as this shows signs of a progressive increase.

This very varied picture of the disease makes assessment of treatment with steroids difficult. There are three ways in which it has been attempted: its effect on mortality rates, in producing diuresis and in improving urine and blood protein levels with prevention of relapses.

This series is not large enough for any statistical comparison of *mortality rates* to be made. It does however make it clear that patients showing early signs of degeneration should be considered separately from the others, that treatment and control groups should be matched for age and that, since probably only a small proportion of adult patients are responsive to steroids, the groups compared must be large.

The *diuresis* obtained by us in some cases with steroid treatment was rapid and accompanied by a decrease in protein excretion and increase in plasma albumen. Similar results have been obtained by many other workers, but there is still uncertainty as to whether this treatment should be used as a routine (17), since diuresis may occur spontaneously or be induced by diuretics such as chlorothiazide or by salt deprivation with ion exchange resins, and since there are dangers in giving steroids. The short courses of moderate dosage which we have used have seldom produced complications and these have not been serious enough to outweigh the advantages of a prompt diuresis and rapid chemical improvement in the cases who respond.

Only some of the patients had this definite response to steroids and no attempt was made in the others to induce diuresis by larger doses. Lange and Wasserman (1) by increasing dosage up to 300 units of ACTH intramuscularly or 30 units intravenously a day, finally obtained a diuresis in all of 41 cases.

sistently for many years. Some of these, even after several years, lose all evidence of disease though only life time studies can show whether such remissions are permanent. Others after varying times during which they may have had periods without symptoms, are seen with terminal renal failure usually preceded by severe hypertension. Oedema in these final attacks is generally less marked than in earlier ones and to some extent due to congestive heart failure, this being a point of difference from the persistent severe oedema seen terminally in most cases with a rapid course.

A very similar picture is given by Johnson and Reader (9) who followed 80 cases of all ages without known complicating systemic disease. Thirteen cases died within two years, often with persistent gross oedema and elevated blood pressure and blood urea from the first attack. Twenty four cases were alive and followed for 2 to 20 years from the first attack, 8 being in complete remission. Two thirds of their cases had raised blood pressure or blood urea or haematuria at the start but this high incidence may be due to failure to distinguish temporary abnormalities which can occur during acute oedematous episodes. In the present series a third of the patients had early hypertension or renal failure leading to death within four years, but this is probably an overestimate of the incidence of acute course cases, owing to selective hospital admission.

A number of papers have recently been published in which classification of cases of the nephrotic syndrome is made from the pathology of kidney biopsy specimens (8, 10, 25, 27). The main division is, into those cases in which the kidney lesion is part of a systemic disease such as amyloidosis, thrombosis involving the renal vein, systemic lupus erythematosus or diabetes, and the rest without known complicating conditions. In the first group the prognosis is bad. Without pathological evidence it is not possible to known whether systemic disease was present in our cases, but it is probable that it was in some of those dying in the early years, especially as the majority of these were seen before the relation of these conditions to the nephrotic syndrome was realised.

The persistence of the condition in a benign form is probably not sufficiently realised, for which two gloomy concepts of the disease are largely responsible. Ellis (3) states that, unless rapid recovery occurs (5 cases out of 145 in his series) there is only one course with steadily progressive renal destruction. But 11 of his 38 cases dying of uraemia lived for 5 to 10 years, one longer and 6 cases were alive after 10 years, 4 after 20. Addis (1) considered that in the majority of cases the nephrotic syndrome is the degenerative stage in nephritis, preceded by an attack of acute nephritis and followed by the final stage of N retention, and characterised throughout by a progressive loss of nephrons.

More recent work, however, gives no evidence that there is any loss of nephrons or irreversible renal damage in many cases with the nephrotic syndrome. Increased blood ureas and decreased filtration rates, in the acute oedematous stage, have been shown by us and by others (5, 7, 13, 15) to return to normal when diuresis has taken place, and the most characteristic sign of abnormal renal function, the high protein excretion, may be recovered from. Even when proteinuria persists it is doubtful whether this indicates continuous disease, Vernier (26) maintaining that it does, but Lange

more studies are needed with repeat biopsies of patients who improve either as the result of steroid treatment or spontaneously

Meanwhile it seems dangerous to withhold steroid treatment from patients with membranous lesions and unwarranted to give a bad prognosis to these who do not respond to steroid, because we do not know in either case, whether the disease will progress.

The recommended procedure, when there is no evidence of renal failure or progressive hypertension, is then as follows. Patients seen with oedema are given a 10—14 day course of ACTH gel, 20 units twice daily or its equivalent. If diuresis does not occur in this time, steroids are stopped and other diuretics used. If good diuresis and reduction in proteinuria occur no further treatment is given unless there is an increase in proteinuria and fall in plasma albumen. These patients, and those who are seen first without oedema, are given 10—30 mg prednisone for 3 consecutive days each week and this, if effective, is continued until the plasma albumen has been normal for at least a month and returned to at any sign of a relapse. If there is no improvement after 3 months no further steroid therapy is given.

Summary

A survey is made of 50 cases of the nephrotic syndrome, followed till death or for 4 or more years from the onset. A group of patients is distinguished in which the disease takes an acute course, hypertension and renal failure developing early and death occurring within a few years. The other patients do not show early failure though temporary mild hypertension and nitrogen retention may be present when oedema is severe. Some of these have a complete remission after one attack of oedema, others after many attacks or years of persistent oedema, others recover except for persistent proteinuria. Relapses are perhaps liable to occur in any patient. Death from renal failure is usually preceded by the development of hypertension.

The difficulties of assessing the effects of steroid treatment, when the disease is so variable, are discussed and three possible lines suggested. A rapid assessment of the diuretic effect of steroids has been carried out. Some patients respond others do not, but the benefit derived by those who do is such that a trial should always be made. Long term treatment with steroids has been judged by comparison of periods with and without treatment in the same patient and here again is very effective in some, of no use in others.

Acknowledgement

I wish to thank Sir Robert Platt and Professor D. A. K. Black, whose patients I have investigated.

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These drastic measures seem dangerous and should perhaps be reserved for the cases who do not respond to other diuretics.

In this series all the patients who had a good response were under 21 years old and those with a poor response older Too much must not be made of this in such a small series and diuresis has been obtained by others in older patients but it suggests that the chances of an effect with steroids decrease with age.

Long term steroid treatment was followed by improvement and absence of relapses in some of the present cases. Since without steroids remissions may occur it is sometimes difficult to be certain that steroids are the effective agents but the prompt, rapid and sustained improvement of some patients, especially those who had previously had frequent attacks of severe oedema is strong evidence of a specific effect.

The dosage used was 5 to 40 mg of prednisone on 3 consecutive days in each week 5 mg was too small to be fully effective. Variations between 10 and 25 mg were made according to progress. 40 mg was only given once to an unresponsive patient and without effect. Lange and Wasserman (12) and Mitchell (16) used 300—400 mg cortisone three times a week. Holland et al (7) found that 5—10 mg prednisolone did not give satisfactory results but 20—50 mg did, and Goodman and Baxter (6) considered 40 mg of prednisone was necessary but that patients refractory to this dose did not respond to larger ones, children seeming to need as large doses as adults. It is perhaps noteworthy and of practical importance that with intermittent dosage at these levels Cushingoid signs did not develop in any patient. Treatment when effective was continued until the serum albumen had been normal for a month at least and in 3 patients for over 4 years. In 2 of these with no proteinuria and minimal proteinuria respectively this was probably unnecessary but the third who has never been free of proteinuria relapsed twice when treatment was stopped. This case illustrates the fact that steroids may be useful even in those cases where proteinuria cannot be abolished. It is also apparent that they may be effective in cases with a long history although it has been suggested that the chances of improvement are reduced if treatment is delayed (12)

Again, only some of the patients showed this improvement with steroids and when after some months the others showed no change, treatment was discontinued. There is not sufficient evidence to show whether it is always those in whom steroids produce a rapid diuresis who are benefited by long term treatment, but this seems probable and if so is a valuable guide to later treatment. Certainly some of those with good diuretic response could only be kept free of oedema with long term treatment On the other hand there are patients who are unaffected by steroids and who do well without, the most striking example being case 47 who has had 3 attacks of oedema, and recovered from the first with no treatment except diet was unaffected by steroids in the second but recovered with resin and is now recovering from the third with resin.

This variation in response to steroids suggests that different forms of the nephrotic syndrome are being treated. Clinically this is not apparent and while kidney biopsy studies suggest that patients with changes of membranous nephritis (type 2 of Ellis) are not responsive while those with proliferative changes are (8, 24 27) this is not yet certain (2 25) and

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The Effect of Acetylcholine on Pulmonary Circulation and Gas Exchange in Cases of Mitral Stenosis

By

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In a previous study Söderholm and Werkö (9) found that acetylcholine given as a constant infusion gave rise to a decreased pulmonary artery pressure and a reduction in arterial oxygen saturation. These findings were interpreted as an effect on the pulmonary vessels, and it was suggested that the shift in blood distribution occurring in mitral valvular disease might be due to an increase in vessel tone in areas of alveolar hypoventilation During acetylcholine infusion, vasodilatation of these areas would cause an increased perfusion of poorly ventilated alveoli and thus decrease in arterial oxygen saturation. Because of lack of data regarding the respiration no definite conclusions could be drawn regarding the mechanism of this shunt like effect

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The present studies were performed in order to further elucidate the effects of acetylcholine on the pulmonary circulation in mitral disease.

Methods and material

The techniques employed were similar to those described earlier (9) A double lumen catheter was positioned to measure the pulmonary artery pressure as well as the pulmonary wedge pressure (PCV) In 8 patients a second catheter was advanced to the right atrium, where the acetylcholine infusion was given. In the other patients the double lumen catheter was withdrawn after the first period to record pulmonary artery pressure in the distal opening, and the infusion was done in the right ventricle

After resting period, basal studies were made in all patients. Pressures were recorded in pulmonary artery (PA) PCV and brachial artery (BA) Cardiac output was measured according to the Fick principle. Expired air was analysed in macro Scholander or a Haldane apparatus. Blood oxygen content was determined with a Beckman B photometer Arterial carbon dioxide pressure was deter

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Table II. Hemodynamic data before and during a constant infusion of acetylcholine at rest

Case		$\bar{P}_{PA}$	$\bar{P}_{PCV}$	$\bar{P}_{B}$	CO	TPR	PVR	SR	$(A-V)_{O}$	V_{O}
K. E.	B	33	16	101	3.8	13.9	9.7	26.6	7.8	300
	D	45	15	103	3.7	12.2	8.1	27.8	7.0	259
L. A.	B	27	17	78	4.3	6.3	2.3	18.1	4.2	181
	D	28	19	82	5.2	5.3	1.7	15.7	3.9	204
N. E.	B	23	11	93	3.8	6.1	3.2	24.5	5.3	204
	D	27	13	101	3.9	6.4	3.1	25.9	5.2	202
K. E.	B	19	13	82	5.4	3.5	1.1	15.1	4.8	259
	D	18	12	83	5.2	3.5	1.1	16.0	4.9	253
A. E.	B	44	30	82	4.4	10.0	3.2	18.6	4.7	207
	D	33	22	77	5.0	6.6	2.2	15.4	4.3	216
J. L.	B	64	27	96	3.7	17.3	10.0	23.9	5.9	220
	D	45	—	94	3.6	12.5	—	26.1	5.5	198
B. W.	B	18	11	127	3.3	5.4	2.1	38.3	6.4	213
	D	20	12	136	3.4	5.9	2.4	40.1	6.4	217
A. L.	B	63	30	86	2.8	22.6	11.8	30.8	8.9	248
	D	66	41	87	3.0	22.3	8.4	29.4	8.3	246
A. B.	B	35	24	82	1.8	19.4	6.1	45.5	6.3	115
	D	41	—	92	2.1	19.5	—	43.8	5.8	122
B. B.	B	24	—	102	—	—		—	—	—
	D	19	—	109	—	—	—	—	—	—
B. G.	B	82	22	121	3.6	27.6	20.0	40.7	7.3	217
	D	78	—	121	5.2	24.1	—	58.3	7.3	231
E. L.	B	38	18	88	4.8	7.8	4.1	18.1	4.7	228
	D	30	20	99	4.5	6.7	2.2	22.2	5.2	232
T. L.	B	53	25	133	4.1	12.8	6.7	32.5	5.4	224
	D	46	—	122	4.6	10.1	—	26.7	5.1	233
S. M.	B	35	27	116	2.3	15.3	3.5	30.6	10.4	238
	D	33	—	113	1.9	17.2	—	59.5	11.9	229
A. A.	B	65	33	147	4.4	14.9	7.3	33.6	5.9	258
	D	60	—	153	3.8	15.7	—	40.5	6.5	249
G. J.	B	55	27	88	5.5	10.4	5.3	16.6	4.7	248
	D	44		79	—	—	—	—	4.1	—
G. S.	B	20	12	104	6.3	3.2	1.3	16.4	4.1	239
N. A.	B	23	19	103	5.0	4.6	0.8	20.6	3.8	190
A. R.	B	24	—	133	3.0	8.1	—	44.3	7.5	222
M. F.	B	20	16	122	6.0	3.3	0.7	20.3	3.5	210
Mean	B	43.6	18.3	101.3	3.7	13.1	4.7	28.9	6.2	222
	D	39.3	19.3	103.3	3.7	12.0	3.7	30.5	6.1	231
P-values for diff.	B–D	<0.02	>0.05	>0.05	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05

Symbols as in table IV

Table I Clinical data

Case	Sex	Age yrs.	Heart volume ml/m	Rhythm	Diagnoses
K. E.	F	48	820	F	M. S.
L. A.	M	48	610	S	M. S.
N E.	M	43	690	F	M S
K. E.	M	55	490	S	M. S.
A. E.	F	59	400	S	M. S.
J I	F	31	480	S	M. S. + Pectus excavatum
S. W	M	53	560	F	M. S op.
A. J	F	36	770	F	M. S.
A. B.	F	32	750	F	M. S.
S. B.	F	43	420	S	M. S. + Bronchiectasis
S. G	F	40	770	F	M. S.
E. L.	F	50	670	F	M. S. + I
T I	F	48	580	S	M. S. + Bronchostenosis
S. M.	F	41	740	F	M S. op. + Tricusp. Insuff.
A. A.	M	54	660	F	M. S. op.
G. J	M	40	600	S	M. S.
G. S.	M	38	790	S	M. I
N A.	F	44	530	S	M. S. + I.
A. R.	F	55	640	F	M. S.
M. F	F	56	520	S	M. S.

M. S. = mitral stenosis
M. S. + I = mitral stenosis and insufficiency
S = sinus rhythm
F = fibrillation
op. = after commissurotomy

mined by a modified Astrup technique (1) Arterial oxygen pressure was calculated from the Dill curve and alveolar P_{O_2} from the alveolar air equation assuming arterial and alveolar P_{CO_2} to be identical. The ratio of venous admixture to arterial blood (Q_V/Q_t) was calculated assuming a post-capillary oxygen saturation of 98 %.

In a second period acetylcholine was infused with a stepwise increase in dosage until 5—10 mg/min. was given. The final dose was adjusted so as to have no effect on the brachial artery pressure and was kept constant for a period of at least 20 minutes. The same recordings as in the resting period were repeated in 16 of 20 patients however PCV pressures were recorded only in 8 patients.

Following the studies at rest 13 patients were studied during bicycle ergometry in the recumbent position. The work performed was approximately 100 kpm/min. Pressure recordings, determination of cardiac output, blood and gas sampling were made between the 5th and 10th minute immediately followed by infusion of acetylcholine in the same dose as at rest. Another set of data was then obtained during the 15th to 20th minute of exercise under continuous infusion.

Table I gives the relevant statistics and diagnosis for the patients. Twenty patients were studied. The usual criteria for mitral stenosis or incompetence have been employed. All the patients were referred to the hospital for evaluation of operability Four were investigated because of restenosis some years after a digital commissurotomy All but two were receiving digitalis at the time of the investigation. Most patients were classified as group II the rest belonging to group III or IV Fifteen cases have been operated and the diagnosis was verified.

Results

Studies at rest

Table II gives the hemodynamic data. It is obvious that acetylcholine in the dose given had no effect on the systemic circulation. Both arterial pressure and cardiac output remain unaltered and thus also the systemic resistance.

Pulmonary artery pressure does decrease significantly but PCV pressure shows only small and inconsistent variations. The two different ways of calculating the resistance in the pulmonary circulation both show a decrease. This decrease would occur by chance less frequently than 5 in 100 times for PVR ($p < 0.05$) and 6 in 100 times for TPR ($p < 0.06$)

Table IV Hemodynamic data during exercise, before and during constant infusion of acetylcholine

Case		$\bar{P}_{PA}$	$\bar{P}_{PCV}$	$\bar{P}_{BA}$	CO	TPR	PVR	SR	(A–V)o	$\dot{V}_{O_2}$
K. E.	B	106	44	121	4.0	26.5	15.5	30.2	17.0	680
	D	96	44	118	4.7	20.4	11.1	25.1	14.2	667
L. A.	B	55	36	85	7.1	7.7	2.7	13.4	7.3	518
	D	59	33	94	8.0	7.4	3.2	11.8	7.6	607
N. E.	B	56	28	114	6.2	9.0	4.5	18.4	8.7	541
	D	44	27	104	5.5	8.0	3.1	18.9	9.6	528
K. L.	B	32	23	87	7.5	4.3	1.2	11.6	8.5	638
	D	28	24	98	8.0	3.5	0.5	12.2	8.4	674
S. W.	B	37	23	137	7.0	5.3	2.0	19.5	12.0	843
	D	37	25	138	6.0	6.2	2.0	23.0	12.3	738
A. B.	B	59	—	92	3.5	16.9	—	26.3	12.0	415
	D	44	—	83	4.0	11.0	—	20.7	10.1	403
S. H.	B	36	—	110	9.6	3.7	—	11.4	6.9	664
	D	29	—	100	6.9	4.2	—	14.4	7.3	507
T. L.	B	82	—	138	3.6	22.7	—	38.2	10.7	386
	D	77	—	149	5.5	13.9	—	27.0	10.5	469
G. J.	B	83	—	90	6.3	13.2	—	14.3	9.6	605
	D	60	—	92	6.7	9.9	—	13.7	9.3	627
G. S.	B	24	19	110	7.6	3.2	0.7	14.5	7.2	547
	D	22	15	113	9.0	2.4	0.8	12.5	6.9	624
N. A.	B	59	46	99	8.5	7.0	1.5	11.7	7.4	627
	D	45	—	101	8.1	5.6	—	12.4	7.6	615
A. R.	B	36	—	135	5.2	6.9	—	25.8	8.1	424
	D	31	—	133	5.4	5.7	—	24.5	8.7	472
M. F.	B	26	17	120	6.9	3.8	1.3	17.3	6.8	470
	D	24	18	122	6.1	3.9	1.0	20.0	6.8	416
Mean	B	53.1	27.1	111.4	6.4	10.0	4.0	19.4	9.4	566
	D	45.8	26.6	111.1	6.4	7.8	3.1	18.2	9.2	573
P-values for diff.	B–D	<0.01	0.05	>0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05

B = before; D = during acetylcholine infusion;

$\bar{P}$ = mean pulmonary artery pressure mm Hg;

$\bar{P}_{BA}$ = mean brachial artery pressure mm Hg

$\bar{P}_{PCV}$ = pulmonary capillary venous pressure mm Hg;

CO = cardiac output l/min;

TPR = total pulmonary resistance $\left(\frac{\bar{P}}{CO}\ \text{mm Hg/l min}\right)$

PVR = pulmonary vascular resistance $\left(\frac{\bar{P}_P - \bar{P}_{PCV}}{CO}\ \text{mm Hg/l/min}\right)$;

SR = systemic resistance $\left(\frac{\bar{P}_{BA}}{CO}\ \text{mm Hg/l min}\right)$;

A–V o_2 = arterio-venous oxygen difference ml/100 ml;

$\dot{V}_{O_2}$ = oxygen uptake STPD ml/min.

Table III. Respiratory data before and during a constant infusion of acetylcholine at rest

Case		Sa_{O_2}	Pa_{CO_2}	V_A	$\frac{V_D}{V_T}$	$\frac{Q_S}{Q_T}$	$(A\text{-}a)_{O_2}$
K. E.	B	97.0	–	–	–	2.5	–
	D	93.6	–	–	–	6.5	–
L. A.	B	98.0	41.0	3.2	34.3	0	8
	D	95.4	39.0	3.1	47.2	9.9	22
N. E.	B	96.2	40.0	3.3	35.7	5.9	8
	D	97.7	33.5	4.0	29.3	0.6	8
K. E.	B	97.0	36.5	4.9	33.2	3.0	10
	D	96.3	34.5	5.1	31.6	4.8	19
A. E.	B	91.0	38.0	3.6	53.2	18.9	36
	D	82.0	41.0	3.7	59.2	40.0	53
J. L.	B	99.0	31.0	4.2	48.8	0	0
	D	93.4	29.0	4.4	46.8	11.3	41
S. W.	B	97.0	41.0	3.6	46.6	3.0	0
	D	98.6	34.0	4.5	38.1	0	0
A. I.	B	94.6	32.5	5.4	50.2	6.5	30
	D	93.4	33.5	4.7	51.0	9.1	33
A. B.	B	96.6	40.0	2.0	38.7	3.8	10
	D	9[illegible].0	43.0	1.9	46.1	8.0	13
S. B.	B	98.5	39.0	4.2	32.5	0	0
	D	96.6	35.0	5.4	28.5	3.3	26
S. G.	B	91.0	34.0	4.8	35.5	10.8	38
	D	92.0	34.5	5.1	38.3	13.9	49
E. L.	B	96.2	37.0	4.2	29.2	5.4	22
	D	98.1	38.0	4.9	33.1	0	5
T. L.	B	92.4	39.0	3.3	44.5	14.1	33
	D	85.9	38.0	3.2	50.5	28.1	47
S. M.	B	93.5	38.0	4.2	42.6	8.6	31
	D	92.7	35.0	4.3	39.2	8.3	38
A. A.	B	88.1	41.0	4.6	55.1	22.8	47
	D	93.7	40.0	4.3	56.3	11.0	36
G. J.	B	98.0	42.0	3.7	41.8	0	0
	D	93.0	39.0	2.5	42.5	*0.7	31
G. S.	B	98.7	–	–	–	0	–
N. A.	B	98.9	37.5	3.5	32.3	0	–
A. R.	B	99.2	38.0	3.8	42.2	0	–
M. F.	B	98.4	43.0	3.0	62.3	0	–
Mean	B	95.4	38.0	4.0	42.9	6.6	17.7
	D	93.7	36.7	4.0	42.5	11.0	28.1
P values for diff.	B–D	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.02

Symbols as in table V

Table III summarizes the respiratory data. Arterial CO tension, V_D/V_T and alveolar ventilation are not influenced by the acetylcholine infusion.

Arterial oxygen saturation decreased on the average by 1.7 % saturation but this change was not statistically significant. In 12 of 16 patients a decrease oc curred and in 6 patients this decrease was more than 2 % saturation. The alveolo-arterial oxygen tension difference increases on the average by 10 mm Hg, which is a significant increase. Venous admixture to arterial blood increases by 4.4 % of cardiac output on the average and this change would occur by chance less frequently than 7 in 100 times ($p < 0.07$)

Studies during exercise

In table IV are presented the hemodynamic data during exercise before and during acetylcholine infusion.

No significant alterations were re corded during acetylcholine infusion in the brachial artery pressure, cardiac out put or systemic resistance.

Pulmonary artery pressure increased markedly during exercise in most cases, and the decrease in PA pressure during acetylcholine infusion is significant. PCV pressure was recorded in 7 patients and remained unchanged. The calculated total pulmonary resistance shows a significant decrease

Table V gives the respiratory data. No significant change occurred in ar terial P_{CO_2}, V_D/V_T or alveolar ventilation. Arterial oxygen saturation decreased significantly

The calculated amount of venous admixture to arterial blood increased significantly as shown by the ratio Q_a/Q as well as the alveolar arterial oxygen tension difference.

Discussion

Pulmonary artery pressure

The frequency with which a pressure drop is elicited during an infusion of acetylcholine has been found to differ depending on whether high or low pressures are found in the pulmonary artery at rest. An arbitrary dividing line has been chosen at 35 mm Hg In this series, among those cases with pressures higher than 35 mm Hg, 8 out of 9 cases did respond to acetylcholine with a decrease in pulmonary artery pressure. Among 7 cases with pressures less than 35 mm, only one showed a response. If to this series of cases are added those of Söderholm and Werkö (9) the same trend is obvious. In cases with pressures above 35 mm Hg 12 out of 13 responded with a decrease in pulmonary artery pressure. Among those within the low pressure group 3 out of 13 cases thus responded. The paper of Bishop et al. (2) shows ex actly the same tendency This observation may be of importance, because in cases with pulmonary disease, no such dividing line has been observed. How ever there are no definite limits within which those who react are found. Both in cases with almost normal pressures and in cases with markedly elevated pressures substantial pressure drops have been observed. Furthermore, among those patients where no reaction was found at rest, a definite decrease in pressure has occurred during exercise and infusion when the pressure level was increased. These results exclude anatomic changes in the vascular bed as the only cause of high pressure in patients with mitral stenosis.

Pulmonary vascular resistance

The difficulties in estimating the pulmonary vascular resistance are obvious.

Table 1. Respiratory data during exercise before and during a constant infusion of acetylcholine

Case		Sa_O	Pa_{CO}	$\dot{V}_A$	$\frac{\dot{V}_D}{\dot{V}_T}$	$\frac{Q_s}{Q_T}$	$(A\text{-}a)_{O_2}$
K. E.	B	95.6	–	–	–	2.9	–
	D	89.9	–	–	–	10.8	–
L. A.	B	97.3	37.5	9.1	30.6	1.5	10
	D	97.3	37.0	8.6	33.3	1.2	2
N. E.	B	94.1	36.0	9.1	22.9	7.3	28
	D	94.5	38.0	8.5	25.5	6.4	27
K. E.	B	95.5	32.5	14.1	15.1	5.1	27
	D	93.9	36.5	13.1	6.6	7.3	31
S. W.	B	98.1	38.0	13.9	29.9	0	0
	D	97.7	35.0	15.1	23.1	0.8	9
A. B.	B	93.6	44.0	7.0	35.6	6.7	26
	D	87.3	45.0	6.7	42.7	16.0	40
S. B.	B	96.7	34.0	16.1	11.5	2.8	27
	D	96.5	35.0	11.5	22.1	2.5	22
T. I.	B	91.0	36.0	7.1	34.4	5.2	36
	D	90.8	37.0	9.7	39.2	9.6	43
G. J.	B	98.0	39.0	11.2	33.1	2.7	0
	D	91.0	36.0	12.1	33.5	8.5	31
G. S.	B	97.9	–	–	–	0.6	–
	D	93.9	–	–	–	9.7	–
N. A.	B	97.9	46.0	10.4	34.6	0.3	0
	D	93.1	43.0	10.9	32.2	5.6	17
A. R.	B	97.6	42.0	6.8	41.8	0.6	0
	D	93.4	38.0	8.4	39.8	8.0	31
M. F.	B	99.2	41.0	8.4	39.7	0	0
	D	99.7	38.0	8.2	37.7	0	0
Mean	B	96.6	38.7	10.3	29.9	2.7	14.0
	D	94.1	38.0	10.2	32.5	6.6	23.0
P values for diff.	B–D	< 0.01	> 0.05	0.05	> 0.05	< 0.01	< 0.05

Sa_O = arterial oxygen saturation per cent

Pa_{CO_2} = arterial carbon dioxide tension mm Hg

$\dot{V}_A$ = alveolar ventilation l/min

$\frac{\dot{V}_D}{\dot{V}_T}$ = dead space ventilation as per cent of total ventilation

$\frac{Q_s}{Q_T}$ = venous admixture to arterial blood as per cent of cardiac output

$(A\text{-}a)_{O_2}$ = alveolo-arterial oxygen tension difference mm Hg.

infusion. Thus our calculations of V_D/V_T are probably correct as are accordingly the estimations of alveolar ventilation. The effect of uneven perfusion on these calculations can be neglected, as the increase in calculated venous admixture during infusion is too small to affect the measurement of arterial P_{CO_2}. It is concluded that alveolar hypoventilation cannot be the cause of the observed decrease in arterial oxygen saturation.

When oxygen consumption, alveolar ventilation and arterial P_{CO_2} are constant, it can be deduced that arterial oxygen saturation must remain unaltered unless the ventilation/perfusion ratio has changed. In this case the only possible explanation would be an increased shunt like effect. This can be expressed either as an increase in venous admixture (Qs/Qt) or as an increased alveolo-arterial oxygen tension difference.

In this series the arterial P_{O_2} was not measured, but has been calculated from arterial oxygen saturation and pH values. In most cases the change in alveolo-arterial oxygen tension difference and the change in shunt was in the same direction and both were significantly increased during acetylcholine infusion. There was no definite relationship between the change in total pulmonary resistance on the one hand and the variation in arterial oxygen saturation on the other nor was the calculated venous admixture or alveolo-arterial oxygen tension difference significantly related to changes in resistance values. These findings do not confirm those of Bishop et al. (2) but it must be emphasized that the test situation was very different in the two investigations. Bishop et al. studied their cases during oxygen breathing while this series was studied during air breathing. Bishop et al. obtained a definite response of pulmonary arterial pressure to oxygen breathing as well as of pulmonary vascular resistance, and the effect of acetylcholine was not marked, while in this series a marked response of pulmonary arterial pressure and resistances was found during acetylcholine infusion. Thus, the two series are not comparable either from a hemodynamic standpoint or in respiratory circumstances. When V_D/V_T decreased, the alveolo-arterial oxygen tension difference tended to increase more than in cases, where V_D/V_T remained constant. This relationship is significantly established.

An increased pulmonary vascular resistance is found in some 25 % of mitral stenosis cases. In those cases studied by Dollery and West (3) with $C^{15}O$ and ^{15}O the distribution of pulmonary blood flow was significantly altered. They found the flow within the upper parts of the lung in the sitting position to be relatively increased. This kind of distribution was more pronounced in cases with manifest pulmonary hypertension. Also pulmonary angiograms have shown narrowing of the basal vessels (8) and pathologists have shown the same distribution of histological changes (4–7).

Dollery and West conclude that the changes in the lower lobes are sufficient in some cases to reduce the lower zone perfusion to a fraction of the normal. The explanation of this change in distribution of perfusion is still obscure!

Pulmonary vascular engorgement, edema etc. is more pronounced in the basal parts of the lung and will result in a decreased compliance of the lung. Overall compliance has been shown to decrease when pulmonary vascular pressures increase (10) and it seems reasonable to assume that the lower parts will be the least compliant and thus less ventilated than the upper parts of the lungs. This

Thus passive alterations of the pulmonary vascular bed (increased pulmonary blood volume or an increase in left atrial pressure) might change the geometry of the pulmonary vessels resulting in a decreased vascular resistance. However a decrease in pulmonary arterial pressure without any change in cardiac output and pulmonary capillary venous pressure indicates a true reduction in pulmonary vascular resistance and a decrease in vascular tone. There has been no correlation between changes in pulmonary arterial pressures or calculated pulmonary resistance on the one hand and systemic resistance or pressure on the other hand. This finding excludes a secondary effect on the lesser circulation due to reduction in the systemic resistance or a shift of pulmonary blood volume. During rest the dose of acetylcholine was slowly increased but during exercise the same dose as the one tolerated at rest was suddenly started in many cases an abrupt change in pulmonary arterial pressure was observed without any change in the brachial artery pressure, and this decrease in PA pressure lasted during the whole period of infusion.

A change in net pressure might occur if the intrathoracic pressure level was altered, e. g by induced bronchoconstriction. In order to get a rough estimate of the intrathoracic pressure variations, the respiratory variations in the PCV recordings were measured. Comparison of these pressure variations before and during acetylcholine infusion did not point to any increase in bronchomotor tone. Thus we think that effects on the bronchi can be excluded as a cause of variations observed in the hemodynamics of the lesser circulation

Nothing is known about bronchopulmonary collateral flow in these cases. However the results reported by others (5) indicate that variations in the collateral flow could hardly be of any significance in the interpretation of our results.

In all cases the total pulmonary resistance was calculated. In those cases where both TPR and PVR could be calculated, these values showed a good correlation ($r = 0.94$) Assuming therefore, that these resistance values would show the same trend of change in all cases, the highly significant decrease in TPR would indicate a change in pulmonary vascular tone.

Arterial oxygen saturation

At rest acetylcholine infusion caused some reduction in arterial oxygen saturation in the majority of cases, but this change was not statistically significant. With exercise, however the same dose of acetylcholine caused a significant decrease in arterial oxygen saturation Several possible mechanisms must be considered to explain this finding

Alveolar hypoventilation can be excluded if oxygen consumption and alveolar ventilation remain unchanged before and during acetylcholine infusion. No significant change appeared in these parameters at rest or during exercise. The calculation of alveolar ventilation and V_D/V_T rests on the assumption that arterial and alveolar P_{CO_2} are identical. In cases of uneven distribution of the ventilation/perfusion ratios this assumption is open to criticism. Acetylcholine might affect the distribution of ventilation through an increased bronchomotor tone. However the respiratory pressure variations in PCV did not show any specific trend, and studies of nitrogen clearance curves in other cases have not shown any changes before and during acetylcholine

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Reversal of Rhythmic Water and Sodium Excretion Patterns during Ganglionic or Adrenergic Block

By

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The usual response of normal persons to a change from the recumbent to the erect attitude is an increased pulse rate and a fall in cardiac output associated with arteriolar constriction and a consequent slight rise in diastolic pressure. The decrease in cardiac output amounts to 20—30 % (McMichael and Sharpey-Schafer 1944, Stead et al. 1945, Lagerlöf et al. 1951) it is explained by a decreased venous return from the lower parts of the body which occurs despite reflex venoconstriction (Sjöstrand 1941, Page et al. 1955). The cardiac output equals or even exceeds its former level when the subject is contracting his calf muscles (Wang et al. 1960). The renal vascular tree takes part in the increase of the peripheral resistance in tilted or quietly standing subjects. Brun et al. (1945) and Smith (1951) found, that the renal plasma flow fell between 20 and 45 % of normal and the glomerular filtration decreased by 20 %, while the mean arterial pressure did not fall.

In standing, sitting, tilted and ambulatory subjects a variable retention of water and sodium (chloride) occurs (Erlanger and Hooker 1904, White et al. 1926, Kattus et al. 1949, Epstein et al. 1951, Ford et al. 1953, Holland and Stead 1954, Newman 1954). As a rule the reduction in the output of water and sodium chloride is accompanied by a fall in glomerular filtration rate, especially during tilting and quiet standing, when excessive demands are being made on the circulation. At least part of this fall is only apparent and results from the effect of the dead space (de Vries et al. 1952). The reduction in glomerular filtration is not an essential factor in the postural antidiuresis and antinatriuresis. In ambulatory subjects an impressive retention of water and sodium may occur without a decrease in glomerular filtration rate (Kattus et al. 1949, McCance 1951, Newman 1954). In subjects, who have resumed the recumbent position after tilting or quiet standing, both the glomerular

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would lead to alveolar hypoventilation which could be one stimulus to the narrowing of the basal vessels The results obtained during acetylcholine infusion are in perfect agreement with this hypothesis.

However this cannot be the only mechanism since

1 No relationship was found between the decrease in pulmonary artery pressure or PVR and arterial oxygen desaturation or calculated shunt during infusion

2 The pressure-response to acetylcholine infusion is more marked in cases with pulmonary artery pressure higher than 35 mm Hg but the calculated shunt was of the same magnitude irrespective of the pressure level

Another possible mechanism is that a chronically increased pressure will induce an increased vascular tone. This has been demonstrated for systemic vessels (6) and would explain our observation of an increased response in the high pressure group The anatomic findings are in agreement with this assumption.

Summary

Acetylcholine infusion in cases of mitral valvular disease during air breathing results in a decrease of pulmonary artery pressure, pulmonary vascular resistance and arterial oxygen saturation

These effects are statistically significant during exercise and the same trend is obvious at rest.

These effects seem to some extent to depend upon the resting level in the pulmonary artery pressure. A decrease in pulmonary artery pressure is more often found in cases with an initially high pressure.

The increased vascular resistance in the pulmonary circulation is supposed to be the result of

1 Regional alveolar hypoventilation due i. a. to alterations in pulmonary compliance.

2 Increased vasomotor tone due to pulmonary hypertension

The decrease in arterial oxygen saturation is explained as venous admixture to arterial blood due to increased perfusion of poorly ventilated areas of the lungs. Other mechanisms are discussed and rejected because of constancy of respiratory data

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only resort for the maintenance of circulatory homeostasis. Administration of saline not only induces a steep rise in blood pressure but also an extremely rapid and enhanced circulatory" diuresis (Wagner 1957) Ten Holt et al. (1952) studied changes in the urinary output of water and electrolytes against the background of the basal diurnal excretory rhythm in a patient with orthostatic hypotension. For several weeks the patient remained in bed and he was given a diet of 8 equal three-hourly feedings daily as is customary in our department for the study of different types of diuresis. The blood pressure promptly rose to hypertensive levels when he was tilted to a 30° head down position, and the blood pressure fell steeply in the reversed position. However both deviations from the normal were self limiting since the hypotension was accompanied by a substantial water and sodium retention and the hypertension by a negative water and sodium balance. Due to the surplus volume of extracellular fluid in the head up position and the deficit in the opposite tilt both the blood pressure and the renal output of water and sodium returned to the control level within 24 hours.

Wagner (1959) states, that almost all patients have a reversal of the normal pattern of urine excretion, manifest by oliguria during the day and polyuria at night. The excessive orthostatic retention of sodium and water was also described by Bachman and Youmans (1953). During tilting relatively small decreases in glomerular filtration rate were accompanied by large decreases in sodium and water excretion rate. On resuming recumbency the glomerular filtration rate and renal plasma flow rapidly returned to the control level, while the rise in water and sodium excretion was delayed. This phenomen was also noted by Wilkins et al. (1953) following temporary venous congestion of the legs in sympathectomized patients with hypertension. As mentioned above it is also found in normal subjects after tilting Thus the renal responses to changes in blood volume and posture are essentially identical in patients with and without adequate vasoregulatory reflexes.

The rare Bradbury Eggleston syndrome can be imitated by means of ganglion blocking agents. In a former investigation we demonstrated in patients with ganglion blockade a correlation of mean" arterial pressure with a gradual change in body position from + 45 to — 45 (Birkenhäger and Statius van Eps 1959) It may be inferred that the arterial pressure will have reflected changes in cardiac performance, and that the atrial filling pressure has changed according to the degree of tilting Thus the validity of Starling's law of the heart can easily be demonstrated in normal human subjects following suppression of vasoregulatory reflexes. Frye and Braunwald (1960) measured cardiac output changes following blood transfusion in normal subjects before and after administration of Arfonad (trimetaphan, used for controlled hypotension in surgery) In untreated patients the cardiac output increased insignificantly (mean increase 0 5 1 per minute) when Arfonad was administered, an impressive rise (average 2.4 l per minute) was noted.

Orthostatic water and sodium retention is enhanced during treatment with ganglion blocking agents (Ford et al. 1953 Grob and McKusick 1953) In recumbent patients Ford et al. noted an appreciable fall in the excretion of water and sodium after intravenous administration of hexamethonium glomerular fil-

filtration rate and the renal plasma flow promptly return to control levels, whilst water and sodium excretion fail to do so for some time and may even temporarily show a further decline (Kattus et al. 1949; Elkington et al. 1951; Epstein et al. 1951). When convalescents have been up and about for several hours, the oliguric phase is followed by a brisk diuresis starting 40 to 60 minutes after resumption of recumbency and a more gradual and prolonged natriuresis (Blomhert et al. 1951; Borst 1954; de Vries et al. 1960).

Both the oliguria and the diuretic reaction form a specific pattern: the changes in excretion almost exclusively involve water, sodium and chloride and to a lesser extent potassium. The reactions are identical in subjects with and without functioning adrenocortical tissue (ten Holt et al. 1952; Chalmers and Squires 1953; Borst 1954; Rosenbaum et al. 1955). Both renal responses represent a universal type of homeostatic reaction which accompanies all changes in circulation. The compensatory effect of the antinatriuresis was first recognized in patients with severe haemorrhage. The water and sodium retention as found in such diverse situations as heart failure, nephrosis and Laennec's cirrhosis, though at first sight not of compensatory character, is regarded as the same type of response on the part of the kidneys, elicited by an "insufficient filling of the arterial system" and favouring the restoration of the normal cardiac performance (Borst 1938, 1948, 1960).

The pathways through which the cardiac performance regulates the renal excretion of "extracellular fluid" are only partly known. Of great importance is the blood pressure in the renal arterial system. In dogs graded narrowing of one renal artery without interfering with the nerve supply was followed by a marked fall in the output of water and sodium of the corresponding kidney before glomerular filtration rate and renal plasma flow showed a measurable diminution (Selkurt et al. 1949; Blake et al. 1950; Mueller et al. 1951). Fluctuations in pressure induced in the renal artery were accompanied by parallel but much more marked changes in the renal excretion of water and sodium, while glomerular filtration rate and renal plasma flow remained almost constant (Selkurt 1949, 1951). Although in normal subjects a fall in blood pressure during standing is prevented by an increase of the peripheral resistance, the kidney reacts to the reduced cardiac performance as if the blood pressure has been slightly reduced. This effect of being ambulatory during daytime is opposed and mostly dominated by the basal diurnal excretory rhythm. Thus most normal people excrete more water and sodium during the day than during the nocturnal period in bed.

When the vasoregulatory reflexes are defective the relationship between venous return, cardiac performance and renal excretion of "extracellular fluid" becomes more overt. In contrast to normal subjects recumbent patients with the Bradbury Eggleston syndrome (1925) respond to moderate reductions in blood volume with an excessive fall in arterial pressure, and to expansion of the blood volume with a rise to hypertensive levels (Wagner 1957). During orthostasis "the filling of the arterial system" is severely defective since both the cardiac output and the arterial pressure are markedly reduced (Hickam and Prior 1951).

In these patients the ability of the kidney to respond to changes in arterial pressure with compensatory changes in the excretion of extracellular fluid is the

Results

In four patients the results were equivocal, either by the occurrence of a marked postural retention of water and sodium in the control period or by an impairment of bladder function (brought about by the parasympathetic blocking action of mecamylamine)

The 3 remaining patients showed a distinct diurnal rhythm in the output of water and electrolytes. Figs. 1—3 illustrate this rhythmical pattern, accompanied by the relatively unimportant fluctuations of the creatinine excretion. In these patients marked diurnal variations in blood pressure were found, which had often a rhythmical appearance. The lowest values occurred in the period after midnight. During ambulation the diastolic blood pressure tended to increase slightly This is in agreement with the results of many former investigations in normal and hypertensive subjects (Asmussen et al. 1939 Foley 1940 Bull 1948 Birkenhäger and Statius van Eps 1959) At the same time the excretion of water and sodium decreased slightly or moderately

On most occasions continuous treatment with mecamylamine or bretylium tosylate caused the excretion of water and sodium to decrease markedly together with a fall in blood pressure, when the patients were ambulatory Sometimes also the excretion of potassium and creatinine decreased. The "dead space" (de Vries et al. 1952) accounts at least in part for the depression in creatinine excretion, as the latter occurred especially during extreme oliguria. As a rule the periods of postural oliguria and sodium retention were followed with some delay by periods of an exaggerated excretion of water and sodium during recumbency

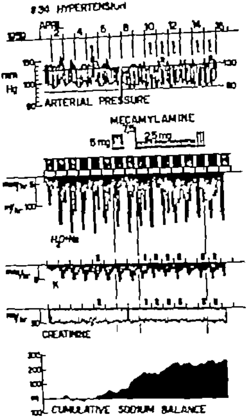


Fig. 2. Patient E. G Strongly marked basal diurnal rhythm in control period. During treatment with mecamylamine postural changes affect both the blood pressure and the pattern of water and sodium excretion. The latter shows the result of postural influences and the effect of basal diurnal rhythm.

One patient (fig 1) permanently confined himself to a sitting position because of bronchial asthma. Treatment with mecamylamine sharply reduced the renal output of water and sodium and suppressed the normal peak of excretion during daytime while potassium excretion continued according to the diurnal rhythm. Reinforced postural oliguria and sodium retention as a consequence of inadequate vascular adaptation apparently is not limited to the vertical stance.

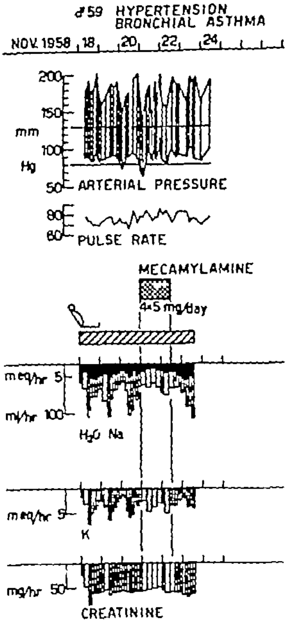


Fig. 1. Patient W. Sitting position in bed was maintained throughout the investigation. During treatment with mecamylamine retention of water and sodium disturbed the rhythmical excretion pattern. Spurious retention because of incomplete bladder evacuation could be ruled out, as potassium and creatinine excretion were only slightly affected.

tration rate and renal plasma flow remained constant though the blood pressure was significantly reduced. The antinatriuresis is not dependent on the presence of functioning adrenal cortical tissue since intravenous injection of hexamethonium induced an identical renal excretion pattern when the production of aldosteron was blocked by amphenone (Lewis et al. 1959).

The present study is concerned with the effects of continuous ganglionic or adrenergic blockade on the diurnal renal excretion pattern in relationship to diurnal fluctuations in arterial pressure. The influence of ambulation and recumbency was studied in the same patients before and during treatment.

Methods

Seven patients with mild to moderate hypertension and who had no evidence of heart failure were studied. During the observation they received the 8 three-hourly equal feedings daily described in previous papers from our group concerning water and electrolyte balance studies (Borst and de Vries 1950, de Vries et al. 1960). The urine was collected in 8 three-hourly portions and examined for sodium, potassium and creatinine. The excretion per hour was recorded. The patients were weighed daily. As a rule the blood pressure and pulse rate were recorded at 0.0 a. m., 3.0 a. m., 8.0 a. m., 11.0 a. m., 2.0 p. m., 5.0 p. m. and 9.0 p. m. After a control period a ganglion blocking agent (mecamylamine) and in one patient an adrenergic blocking agent (bretylium tosylate) was administered orally at 3- or 6-hourly intervals. The effects of ambulation were studied during periods of 3—6 hours, synchronized with the above mentioned 3-hourly periods (mostly 9 a. m. — 3 p. m.). These periods are indicated by black rectangles. When they were not ambulatory the patients during the day (6 a. m. — 9 p. m.) were in bed in a semirecumbent position, as indicated by hatched rectangles. An exceptional period of prolonged strict recumbency during daytime has been recorded in fig. 3. During the night (9 p. m. — 6 a. m.) all patients but one (fig. 1) were strictly recumbent (blank rectangles).

decline of creatinine excretion, the virtual weight gain during the observation was a good 1 kg.

When patient E. G (fig 2) was up and about during the control period, the sodium balance became slightly positive. Treatment with a high dose of mecamylamine induced a marked hypotension, associated with a steep rise of the cumulative sodium balance, which rise continued the following day On day 9 after a "priming dose" of 7.5 mg treatment was continued with 8 doses of 2.5 mg mecamylamine daily In this period both blood pressure and sodium excretion decreased only during ambulation. The cumulative sodium balance became more positive for the third time, when the patient was up and about for 6 hours (days 10 and 11) thereafter a new equilibrium was established, the retained quantity of sodium being excreted in the early morning hours (3 to 9 a.m.) This suppression and retardation of nocturia, occurring even when the orthostatic oliguria during day time entails a rise of blood pressure in the evening, may be supposed to be inherent in the very marked basic diurnal rhythm in this patient. In all, 210 mEq of sodium were retained during the period of ganglionic block.

In patient E. S. (fig 3) the spontaneous postural retention of water and sodium was partly corrected by a marked diuresis during a day of bedrest (day 20) On this day treatment with bretylium tosylate was started. The low initial dose was meant as a test for idiosyncrasy and no effective adrenergic block was expected. Nevertheless a mild adrenergic block ing effect may have enhanced the excretion of the retained water and sodium during bedrest. Consequent to the loss of extracellular fluid the blood pressure decreased in the recumbent patient, before the dose of bretylium tosylate had been increased.

A marked oliguria and sodium retention occurred on day 22 when the patient was ambulatory for 6 hours. An impressive nocturia followed, with a peak between 9 p. m. and midnight. This postural reversal of the diurnal excretion pattern was repeated during the following days. It is obvious that after the initial increase a further rise of the cumulative sodium balance was prevented by the nocturnal diuresis. The prompt and very marked nocturia and the relatively weak basic diurnal rhythm, as found in the control period, are probably connected. The blood pressure attained its maximum at 9 p. m. At midnight it tended to surpass the level found in the control period. Meanwhile a certain degree of "tolerance" against the hypotensive action of the drug seemed to occur On days 25—27 when the patient was strictly recumbent for 33 hours, a large amount of water and sodium was excreted and the cumulative sodium balance returned to the base-line. The blood pressure with some retardation paralleled the cumulative sodium balance. A remarkably severe orthostatic hypotension was noted the following day suggesting that the above-mentioned tolerance had been due to the accumulation of saline. Thereafter a sodium retention was observed and the accumulation of sodium progressed during the periods of ambulation.

The postural hypotensive and salt retaining effect of bretylium tosylate lasted at least one and possibly two days after the treatment was stopped. On the subsequent days sodium intake and output were in balance the excretion of superfluous sodium was apparently counteracted by the moderate sodium retaining effect of ambulation. The administration

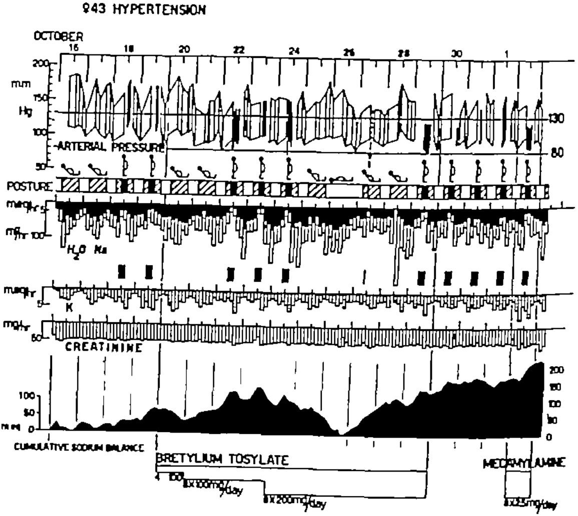


Fig 3 Patient E. S Postural changes of blood pressure and sodium and water excretion during adrenergic block are identical with those during ganglionic block.

In two patients (figs. 2 and 3) an approximate cumulative sodium balance was calculated. Similar experiments in our department have shown that on the average a loss of 12 mEq of sodium daily is unaccounted for This is due to such factors as spilling of slight amounts of beverage and urine, incomplete consumptions of rations, and excretion of sodium in sweat and stools. In our patients this estimate appears to be correct, since the cumulative sodium balance which was thus corrected, fluctuated rhythmically along the base line while the patients were in bed during the control period Although the patients were weighed daily the body weight curves are not recorded in figs. 2 and 3 since a correction had to be made for a gradual loss of weight throughout the observation period. Thus the tendency to put on weight during phases of a positive sodium balance did not result in an ultimate weight gain that corresponded with the accumulated amount of extracellular fluid" Presumably this was due rather to muscular atrophy (caused by sharply diminished physical activity) than to a caloric deficit. When a correction was made for the loss of muscle mass as estimated from the

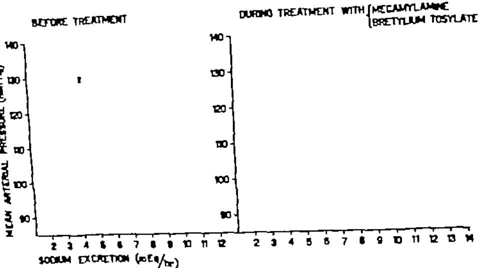


Fig. 4 Patient E. S. Mean arterial pressure and sodium excretion are plotted, regardless of posture or diurnal period. A correlation was found only during adrenergic or ganglionic block. The range of sodium excretion nearly doubled, by expansion in both directions.

tion of vasoregulatory reflexes the kidney promptly corrects excessive as well as insufficient filling of the arterial system.

The correlation between mean arterial pressure and renal sodium excretion, as shown in the second part of fig 4 reminds one of the observations of Selkurt on the effect of fluctuations in pressure in the renal artery on the renal output of sodium and water It is highly improbable, that a mechanism like this would only operate when adrenergic stimuli are blocked or — like in Selkurt dogs — are kept constant Fig 3 shows that before treatment also and 3 days after treatment renal sodium output is conditioned by posture in spite of the absence of correlation between mean arterial pressure and sodium excretion as demonstrated by the first part of fig 4 The range of sodium excretion in the untreated patient is, however only about half of that found during ganglionic or adrenergic block, the mean excretion being the same, viz. 7 mEq/hour Apparently the effect of posture on renal sodium excretion is buffered by vasoregulatory reflexes while the effect on systemic blood pressure is almost completely annihilated.

The positive shift of the (approximate) cumulative sodium balance during ganglionic or adrenergic block, being largely a consequence of postural sodium retention, is of practical importance. When the sodium accumulation is progressive, the circulatory system will be over-burdened. During treatment with pentolinium (Ronnov-Jensen 1955) and guanethidine (Dollery et al. 1960 Ferting et al. 1961) patients indeed have developed congestive heart failure, especially when they had a history f heart failure. Fortunately such a development is exceptional. In most cases sodium retention is limited, probably because of an effective circulatory homeostasis. Ford et al. (1953) noting that none of their patients exhibited oedema or other signs of fluid retention in spite of the pparently depressed excretion of water and electrolytes, suggested that the augmented excretion during the sleeping period, as observed in one patient, accounted for the reestablishment

of mecamylamine induced a further sodium retention. In all 200 mEq of sodium were accumulated. The relationship between blood pressure and sodium excretion in this patient is demonstrated in fig. 4.

Discussion

The rhythmical appearance of the diurnal blood pressure pattern in the control period, as found in 3 patients, could at first sight be associated with the diurnal rhythm of water and electrolyte excretion. Fig. 4 however shows that in the untreated patient a correlation between the calculated mean arterial blood pressure and sodium excretion may be completely absent.

During incomplete recumbency the effect of ganglionic block on the excretion of water and sodium is apparently unpredictable. Slight postural changes and physical activity are likely to induce exaggerated variations in urine flow and sodium excretion, as appears from fig. 3. One patient was accustomed to a sitting position because of anxiety neurosis and bronchial asthma (*fig. 1*). This habit was reflected in a significant retention of water and sodium during moderate ganglionic block. In patient E. G. (fig. 2) treatment with a hypotensive dose of mecamylamine induced a marked retention of water and sodium although she was not ambulant. Thus it appears that ganglion blocking agents in *high* dosage may effect a retention of water and sodium during bedrest, probable due to their hypotensive action; sitting propped up in the cushions will accentuate this effect by impeding the venous return.

The effects of *moderate* ganglionic or adrenergic block are completely different during ambulation and during bedrest. During ambulation these drugs are bound to induce oliguria and sodium retention, presumably as a consequence of the decline in blood pressure. The latter results from a decrease in venous return (and consequently a decrease in cardiac performance) together with a defective adjustment of the arterial system. During bedrest the arterial pressure rises, probably as a consequence of the failure of vasoregulatory reflexes to prevent an increase of cardiac output (Frye and Braunwald 1960). The renal response to the increase in blood pressure consists of a selective diuresis of water and sodium. The nocturnal "circulatory" diuretic stimulus has to break through the oliguric phase of the endogenous diurnal rhythm. In patients with a relatively weak basic diurnal excretory rhythm the nocturnal diuretic response can be very marked (fig. 3). Here a reversed diurnal excretory pattern comes into existence. Yet the endogenous diurnal rhythm persists, as appears from potassium excretion. In patients with a strong basic rhythm the nocturnal diuretic response may be suppressed (fig. 2, day *10—11*) or delayed to the early morning hours (days *12—15*). This modification of the "circulatory" diuresis by the basic diurnal excretory *rhythm has been described by* ten Holt et al. (1952), Borst (1954) and de Vries et al. (1960). The homeostatic effect of the circulatory diuresis during day time, when the latter is reinforced rather than encumbered by the endogenous diurnal rhythm, is obvious in fig. 3 (days 25—26). The marked diuresis corresponding to a phase of increased blood pressure in its turn obviously brought the phase of hypertension to an end. In this period the blood pressure pattern almost exactly paralleled that of the cumulative sodium balance. Apparently during the inhibi-

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of fluid balance. Our observations confirm this suggestion, the accumulation of sodium being limited to about 200 mEq in the first 3—5 days. A moderate weight gain (about 1 kg) is observed in many patients who are treated with ganglionic blocking agents or with guanethidine (Ronnov-Jessen 1955, 1960, 1961; Leishman et al. 1959; Dollery et al. 1960; Fertig et al. 1961). Most authors identify this weight gain with fluid retention although none refers to its mechanisms as discussed by Ford et al. (1953). Ronnov-Jessen (1960, 1961) actually demonstrated in 12 of the 14 patients after a few days of treatment an increase in blood volume (on the average 7.9 %). A close correlation with body weight changes has not been found. The following factors may be expected to determine the extend of sodium accumulation: the intensity of postural sodium retention, the duration of ambulation, the cardiac reserve and the strength of the endogenous diurnal excretory rhythm. Even a limited sodium accumulation is of practical importance since it may entail drug tolerance, as in case E. S. (fig. 3). The necessity to preclude this by restriction of salt intake and administration of diuretics now becomes widely recognized.

Summary

The effect of posture on water and sodium excretion during continuous treatment with a ganglionic or adrenergic blocking agent was studied in 3 hypertensive patients against the background of the basal diurnal rhythm of water and electrolyte excretion.

During treatment orthostasis was almost invariably accompanied by a decrease in blood pressure and by a much more marked water and sodium retention than before treatment. The depression of potassium and creatinine excretion was either absent or less obvious than that of sodium. In the following night the greater part of the retained water and sodium was excreted, provided the patient was recumbent and the endogenous diurnal excretory rhythm was not too vigorous. This selective diuresis was as a rule preceded by a rise in arterial pressure; hence both the diurnal excretory pattern concerning water and sodium and the diurnal blood pressure pattern could be reversed.

No correlation between mean arterial pressure and renal sodium output was found under physiological conditions; however, in a patient in whom vasoregulatory reflexes were suppressed by mecamylamine or bretylium tosylate during 11 days a correlation between the two parameters could be clearly demonstrated (fig. 4). In this condition circulatory homeostasis mainly depends on the renal response to alterations in blood pressure. In two patients a cumulative sodium balance was calculated during treatment: both retained about 200 mEq of sodium before equilibrium was re-established. Drug tolerance can be explained by this accumulation of saline.

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The Presence in Mammalian Liver and Blood of Substances which Interfere with the Growth of Vaccinia Virus

I. Methods for Preparing Biologically Active Materials and their Chemical Character

By

P. Laland, J. Dedichen, S. Laland and N. Thorsdalen

Our interest in liver started with the work on the "antianemic principle" (1—5) and the leukocytosis factor (6, 7). In connection with further work on the leukocytosis factor we submitted suitably prepared liver fractions to a number of biological assays. During this work it was discovered that liver contains a substance(s) which interferes with the growth of vaccinia virus in chick embryo and rabbit' skin and increases the resistance to E. coli infection in mice. Similar substances have also been discovered in blood. The present report describes methods for preparing materials exhibiting such activities. In addition some analytical data on such materials are reported. The biological activities of these materials are described in the following paper.

Thus a research work which started in search of a substance of importance to the normal development of the white blood cells has ended in the preparation of material capable of protecting against certain types of infection.

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Methods

Preparation of liver paste

10.7 kg of ox liver (straight from the animal) was homogenized in a Waring blender in water (20 l) containing 0.34 % tricresol (freshly distilled water was used here and in all subsequent stages) and the mixture left for three hours at room temperature. It was then heated[1] to 9[illegible] for 30 min., left for 24 hours at room temperature and filtered. The filtrate was concentrated at 60—70 in vacuo to a syrup (700 g). To this which contained 405 g of dry solid was added 75 % ethanol (3.15 l) and the mixture left three days at room temperature. The precipitate was filtered off, washed with 70 % ethanol (800 ml) and the filtrate concentrated at 30—40 under nitrogen in vacuo to a syrup (275 g) which contained 194 g of dry solid.

[1] All temperatures are given in degrees centigrade.

Table I. Flow sheet for preparing biologically active materials from liver

Liver homogenized in water and heated at 100	
Filtrate concentrated	
Ethanol to 70 %	
Filtrate concentrated	
Aqueous solution of liver paste	
Extracted into 90 % phenol	Treated with asbestos
Ether added and liver material re-extracted into water	Asbestos eluted with 100 % phenol at 60°
Barium acetate and Ethanol added	Phenol eluate precipitated with ether
Precipitate redissolved in water and passed through Amberlite IR 120 in the hydrogen form	Precipitate Material III
Effluent concentrated Material I	
Fractionated on Sephadex G 50, high molecular fraction collected Material II	

acid-water (25 6 25 v/v) and n-butanol-methyl ethyl ketone-water (2 2 1 v/v) in an atmosphere of cyclohexylamine (1 ml for every 25 ml of solvent mixture). The chromatogram was dipped in 0.25 w/v solution of ninhydrine in acetone containing 7 glacial acetic acid and finally heated for 15 min at 45

Carbohydrate analysis

About 2 mg of material was hydrolyzed in 2 N-H_2SO_4 (0 2 ml in sealed tube for 3 hours 100 The hydrolysate was neutralized with $BaCO_3$ the supernatant removed after centrifuging, freeze-dried and the residue redissolved in water (0.1 ml). Aliquots were applied to Whatman no. 1 paper and the strip developed with the solvent mixture n-butanol-water-pyridine (3 2 1.5) (9). The chromatogram was sprayed with aniline phthalate and heated at 105 for 5 min.

Table II. The phosphorus content of materials prepared from liver and blood

Materials	Phosphorus %
II (from liver)	0.03
III (from liver)	0.50
IV (from blood)	0.035

Results and discussion

All the materials described protect against vaccinia virus in chick embryo and in rabbit's skin and against E. coli in mice (see the following paper). A flow sheet of two alternative methods for preparing such materials from liver is seen in table I. The methods of preparation show that the active substance(s) is soluble in 100 phenol and that its molecular weight is equal or greater than 10[4] since it appears in the fractions immediately after the void volume on a Sephadex G 50 column (11). The solubility in 100 phenol is interesting and in sharp contrast to the insolubility in phenol of such high molecular material as bacterial lipo-polysaccharides (12). The active material is adsorbed onto asbestos. Furthermore it tolerates heating at 100. Material II and III exhibit a characteristic green-yellow fluorescence in the ultraviolet light and give a precipitate with phosphotungstic acid.

Material IV the active material from blood, resembles that from liver since it has a molecular weight equal to 10^4 or greater tolerates heating at 100° exhibits a yellow-green flourescence in ultraviolet light and gives a precipitate with

The authors have prepared from zymosan water soluble substance which interferes with the growth of vaccinia virus and increases the resistance to E. coli infection in mice. It is probably polypeptide and exhibits green-yellow fluorescence

Two alternative methods of producing biologically active materials from liver paste are described. In one case the starting material was commercial liver paste

Preparation of materials I and II

420 g of liver paste (Liver Fraction I Paste N F from Armour Pharmaceutical Company Chicago) was mixed with water (3 l) and the solution extracted 8 times with aqueous 90 % phenol 350 ml each time The combined extracts were mixed with ether (17.5 l) and water (500 ml) and the aqueous phase separated off the next day The ether phenol phase was subsequently extracted twice with water (350 ml each time) and the combined solutions concentrated in vacuo. To the concentrate (1 l) was added tricresol (0.75 g) and the pH adjusted to 8.25 by the addition of 10 KOH Barium acetate (27 4 g) was then dissolved, and 96 ethanol (4 4 l) added. Next day the supernatant fluid was decanted off from the sticky precipitate which was then dissolved in water (600 ml containing 0 3 tricresol) and passed through a column of Amberlite IR120 (6 cm diameter ×40 cm) in the hydrogen form (the column was washed before use with water containing 0.3 tricresol) followed by water (containing 0.3 tricresol) until the effluent was colourless. The acidic effluent was neutralized with ammonia concentrated in vacuo and finally freeze-dried The dark material obtained weighed 20 g (liver material I)

Material I (10 g) was dissolved in water (40 ml) and fractionated at + 4 on a Sephadex G 50 column (4.5 cm diameter × 30 cm void volume 70 ml) The first 70 ml (void volume) was discarded and the next 200 ml was freeze-dried and yielded 150 mg of a brown material (liver material II)

Preparation of material III

Liver paste (27 5 g not commercial) was dissolved in water (110 ml containing 0 3 tricresol) and asbestos (0 56 g British Drug House) added. The mixture was stirred at room temperature for 24 hours, the asbestos isolated after centrifuging and washed with water (25 ml) The washed asbestos was dried in a vacuum desiccator at room temperature extracted at 60 with crystalline phenol (20 g) for 24 hours. The supernatant was removed after centrifugation and the asbestos reextracted with phenol (20 g) in a similar manner To the combined phenol extract was added ether (200 ml), the precipitate isolated by centrifuging and washed with ether It was dissolved in water pH adjusted with 0 1 N NaOH to 7 0 and the solution freeze-dried The yellow brown material weighed 68.2 mg (liver material III). Biologically active material can also be eluted from the material with 0 1 N NaOH.

Preparation of material IV

To fresh ox blood (5 l) was added tricresol (12.5 ml) the mixture heated under stirring at 100° for 3 hours and filtered. The coagulated protein was washed with water (0.5 l containing 0.3 tricresol) To the filtrate (3.8 l) was added an aqueous solution of 20 % sulphosalicylic acid (25.5 ml) and the precipitate removed by filtration. The filtrate was concentrated in vacuo under nitrogen at 30—40 pH adjusted to 8.0 by the addition of 7.5 N NaOH and the cloudy solution filtered The filtrate (70 ml) was passed through a column of Sephadex G 50 (4 cm diameter × 32 cm, void volume 100 ml) equilibrated with 0 01 N NaOH containing 0 3 % tricresol. The first 80 ml of effluent was discarded. The next 153 ml exhibited a green-yellow fluorescence was neutralized by the addition of Amberlite IR120 in the hydrogen form, tricresol removed by extraction with ether and the solution freeze dried The nearly colourless material weighed 482 mg (material IV)

Estimation of total phosphorus

A slightly modified method of that described by Jones et al. (8) was used. The amount of the materials analysed varied from 3—15 mg

Qualitative amino acid analysis

About 1 mg of material was hydrolyzed in a sealed tube at 110 in 6 N HCl (0 1 ml) for 4 hours. The hydrochloric acid was removed at 60° When dry water (0.1 ml) was added, the solution again taken down to dryness and the residue kept over night in vacuo over KOH The residue was dissolved in water (0.1 ml) and an aliquot submitted to two dimensional chromatography according to the method of Miz ll and Simpson (9) on Whatman no. 1 paper The solvent systems used were n-butanol-acetic

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The Presence in Mammalian Liver and Blood of Substances which Interfere with the Growth of Vaccinia Virus

II. Testing of Materials

By

J. Dedichen, P. Laland, S. Laland and J. Voss

In this work the effect of materials prepared from liver and blood (described in part I) on chick embryos challenged with vaccinia virus, on the development of vaccinia in the rabbit's skin and in children has been studied. In addition the ability of the preparations to increase the resistance of mice challenged with E. coli is reported. Pyrogenicity and antigenicity tests on the preparations are also presented. Some of these experiments have been published in two preliminary reports (1, 2).

Methods

Vaccinia virus

The vaccinia virus used was vaccine T166 prepared from cell culture of calf embryo epithelial cells infected with vaccinia virus and was free of bacteria. In some experiments ordinary calf lymph vaccines (B32 and B33) with content of bacteria of less than 600/ml were used.

Virus test in chick embryo

Eggs from White Leghorn were used. The eggs were incubated for 10 days at 37°, a 0.5 cm × 0.5 cm window was drilled over the chorio-allantoic membrane and the membrane dropped by suction over the air sac. The solution to be tested was applied to the membrane. The vaccinia virus was applied on the same spot either simultaneously or after incubation for 24 hours at 37°. The window was sealed with cellophane tape and the eggs incubated for four days after inoculation. The eggs were broken in Petri dish, deaths determined and the pocks produced by the vaccinia virus estimated as a score according to the following key:

Growth of virus	Score
No growth	0
< 20 pocks	1
> 20 scattered pocks	2
Numerous, non confluent pocks	3
Confluent pocks	4

All temperatures are given in degrees centigrade.

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phosphotungstic acid. We have also shown that the active material from blood is adsorbed onto asbestos.

Material II, III from liver and material IV from ox blood were analysed for total phosphorus and the results are seen in table II. All fractions contained only traces of phosphorus. Thus, if present, there would be less than 0.5 % of a nucleic acid (except in material III).

The following amino acids were found in materials II, III and IV after acid hydrolysis. Aspartic acid, glutamic acid, glycine, alanine, serine, arginine, lysine, valine, leucine, isoleucine and phenylalanine. Histidine was present in small amounts in materials III and IV. Traces of methionine and threonine were present in material IV. Hydrolysate corresponding to 0.4 mg material was put on the chromatogram.

The absence of such common amino acids as tyrosine, proline, methionine, threonine and cysteine is apparent. However, the destruction of some of these amino acids during hydrolysis could account for their absence. That this was probably not the case was shown by the fact that in a hydrolysate consisting of an equal mixture of albumin and material II tyrosine, proline, methionine, threonine and cysteine could be detected.

None of the ordinary monosaccharides were detectable after acid hydrolysis in the two materials described. Small amounts of galactose, however, were present in the material prepared from blood. The presence of a polysaccharide as a major component of any of the materials examined does therefore not seem a very likely possibility.

Summary

Two alternative methods for producing purified material from liver which will interfere with the growth of vaccinia virus and increase resistance to E. coli infection in mice are described. A method for producing such material from blood is also reported. The active material(s) has a molecular weight equal to or greater than 10⁴, exhibits in ultraviolet light a green-yellow fluorescence, contains after acid hydrolysis about 12 of the common amino acids. After acid hydrolysis only the material from blood was shown to contain small amount of carbohydrate. The biologically active preparations contain only trace amounts of phosporus.

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Table I The effect of the administration of material I (from liver) to chick embryos 24 hours before infection with vaccinia virus (T166) 0.05 ml containing 62.5 µg material was given to the embryos

Dilution of vaccinia virus	Survivors/total embryos		Growth of virus (score)	
	Treated	Un-treated	Treated	Un-treated
10^{-5}	5/7	0 7	14	28
10^{-6}	5 5	2/5	10	20
10^{-7}	6/6	6/6	8	12

Material I was dissolved in pyrogen free saline and filtered through sintered glass filter G5. Before use it was diluted 1 100 with saline.

Table II The effect of the administration of material II (from liver) to chick embryos 24 hours before infection with vaccinia virus (T166) 0.05 ml containing 2 µg of the material was given to the embryos

Dilution of vaccinia virus	Survivors/total embryos		Growth of vaccinia (score)	
	Treated	Un-treated	Treated	Un-treated
10^{-5}	5 6	1 6	12	24
10^{-}	6 6	0 6	6	10
10^{-}	6 6	6 6	6	12

Material II (from liver) was dissolved in pyrogen free water containing 0.3 tricresol followed by filtration through sintered glass filter G5. Before use was diluted 1 100 with saline.

Table III Control experiment in chick embryos Pyrogen free water containing 0.3% tricresol was diluted 1 10 with saline and 0.05 ml administered 24 hours before infection with vaccinia virus (B33)

Dilution of vaccinia virus	Survivors/total embryos	Growth of vaccinia virus
10^{-6}	0/6	Confluent growth
10^{-7}	0/6	Confluent growth
10^{-8}	0 6	Confluent growth

18 embryos received 0.05 ml of the solution but no vaccinia virus, and all embryos were alive after four days.

Table IV The effect of the administration of material III (from liver) 24 hours before infection with vaccinia virus (B32) 0.05 ml containing 37 µg of the material was given to the embryos

Dilution of vaccinia virus	Survivors/total embryos		Growth of vaccinia (score)	
	Treated	Un-treated	Treated	Un-treated
10^{-5}	5/5	0 5	10	20
10^{-6}	5 5	0 5	5	15
10^{-7}	5 5	1 5	5	15
10^{-8}	5 5	5 5	0	15

Material III (from liver) was dissolved in pyrogen free water containing 0.3% tricresol. Before use it was diluted 1 10 with saline.

THE EFFECT OF THE MATERIALS ON RABBIT'S SKIN INOCULATED WITH VACCINIA VIRUS

First test on rabbit's skin with material I

Vaccinia virus (T166) was used. A solution of material I (from liver) (62 mg ml in pyrogen free water containing 0.3 tricresol was diluted 1 10 with saline 0.5 ml was injected subcutaneously into one rabbit. The rabbit was inoculated one hour later with vaccinia virus at the injection site and at various points these being 1 2 3 4 and 6 cm respectively (the inoculation sites being about symmetrically distributed around the site of injection of the material) from the site of the injection of the material. The results were read after five days. No lesions developed at the site of injection of the material or at the 1 cm inoculation site. Otherwise lesions developed around the remaining inoculation sites.

Virus test in rabbit's skin

White land rabbits weighing 3—4 kg were used. The animals were epilated with barium sulphide on the flanks. 0.5 ml of the solution to be tested was injected intracutaneously or subcutaneously In some experiments the inoculation with vaccinia virus was made by injection at the injection site of the test material In other experiments the inoculation of virus was made on a 3 cm × 3 cm scarified area over the site of the injected material.

Challenge of mice with E. coli

Swiss albino mice weight 14—22 g have been used throughout. Treated animals were separated from the controls.

A strain of E coli obtained from Glaxo Laboratories Greenford England, was grown in phosphate broth containing 10 serum for 24 hours at 37 and diluted with the medium to give the concentration of the organisms required The number of bacteria (3) was estimated by the light absorption at 650 mμ in 1 cm cells in a Beckman Spectrophotometer DK assuming a 1 ml suspension of optical density 0 30 to contain 10^9 cells. 0.25 ml of the bacterial suspension was injected by the intraperitoneal route 0 1 ml of a solution of the material to be tested was injected mainly by the intraperitoneal route In some of the experiments, material was injected into one of the tail veins and in others intramuscularly

Zymosan according to Howard et al. (4) increases the resistance to E. coli infections in mice Zymosan has been a useful tool in securing that the E. coli challenge is of a magnitude which provides conditions for unveiling differences in the non-specific resistance 0 1 ml of a suspension of zymosan (Ex. fresh yeast, type A from L Light & Co. Ltd Colnbrooke England) in saline (1 mg/ml) was injected intraperitoneally

Antigen test

1 ml of the solution to be tested was injected intramuscularly in healthy young volunteers. One week after the intramuscular injection the solution was injected intracutaneously The skin reaction provoked was compared with the reaction of the intracutaneous application of a solution of 0 1 histamine and to that of a solution of the test material diluted 1 1 000. The reactions were read after 20 min.

Pyrogen test

Himalayan rabbits weighing 1.5—2.5 kg were placed in boxes and the rabbits kept in position by a guard around the neck. The thermocouples were inserted 7.5—10 cm into the rectum The temperature was read three times before injecting the rabbits. If the two last readings did not differ by more than 0.2 the rabbits were injected intravenously The rectal temperatures were then read every 15 min. for the first hour and later every 30 min. for 3 hours.

Results

THE EFFECT OF THE MATERIALS ON CHICK EMBRYO CHALLENGED WITH VACCINIA VIRUS

The effect of the administration of material I (from liver) to chick embryos challenged with vaccinia virus is seen in table I It is seen that material I interferes with the growth of the virus. Table II presents results in the chick embryo system with material II which was derived from material I upon purification (see part I p 118) As indicated in table II only 2 μg material per embryo is sufficient to interfere with the growth of the virus. Since the solution of material II contained small amounts of tricresol a control experiment described in table III was carried out. The amount of tricresol used had no protective effect

In table IV the effect of material III (from liver) in the chick embryo test is shown The dose used per embryo was 37 μg which produces a considerable degree of protection.

It is also seen from table V that 4.8 μg of material IV (prepared from blood) produces significant protection against vaccinia virus in the chick embryo



Fig. 1. The effect of material I (from liver) on the development of vaccinia virus lesions in the rabbit's skin. From the left the inoculated areas are designated A, B, and C respectively

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Table I The effect of the administration of material II (from blood) to chick embryos 48 hours before infection with vaccinia virus (B32) 0 05 ml containing 9.6 µg of the material was given to the embryos

Dilution of vaccinia virus	Survivors/total embryos		Growth of virus (score)	
	Treated	Untreated	Treated	Untreated
10	5/5	0/5	10	10
10⁻	5/5	0/5	5	15
10	5/5	3/5	5	15

Material IV was dissolved in pyrogen free water containing 0.3 % tricresol. Before use it was diluted 1 10 with saline

In another experiment one rabbit was inoculated with the vaccinia virus (T166) by scarification at three different sites 0.5 ml of the diluted solution of material I was injected into each of the three areas respectively The result after 6 days is seen in fig 1 In A the injection of the material was immediately after the inoculation in B and C 24 and 48 hours after respectively It was found that material I interferes with the growth of the vaccinia virus when injected at the same times as the inoculation of the virus

Virus test in rabbit s skin with material III

0 5 ml of a solution of material III (from liver) (0 4 mg/ml) in water containing 0 03 % tricresol was injected intracutaneously into each of two rabbits Rabbit no. 1 received simultaneously inoculation with vaccinia virus (B32 undiluted) by scarification and rabbit no 2 was inoculated 24 hours after the injection. The reactions were read after 6 days In rabbit no 1 a 1 cm × 1 cm area was free of lesions and in rabbit no 2 the area was 1.5 cm × 1 cm

Table II The effect of the administration of material I (from liver) to children prior to or simultaneous with the inoculation of vaccinia virus (corpus vaccine) Thirteen children were given in the left arm intracutaneously 0 1 ml (12.5 mg) of a dilute solution of material I Six of the children were inoculated immediately after on the same site with the vaccine and the untreated right arm was inoculated as a control The remaining 7 children were inoculated with vaccine on the site of the injection of material I on the left arm 24 hours later and on the right arm as a control. The results were recorded at days later

Child no.	Time (hours) between injection of material and inoculation	Reaction on right arm	Reaction on left arm
1	0	+	weakly +
2	0	++	+
3	0	++	+
4	0	++	+
5	0	++	+ (−)
6	0	++	+
7	24	+	−
8	24	+	−
9	24	+	−
10	24	+	−
11	24	+	weakly +
12	24	++	−
13	24	+	−

\+ = positive
− = negative.

Virus test in rabbit s skin with material IV

0 5 ml of a solution of material IV (from ox blood) (0 096 mg/ml) in water containing 0 03 tricresol was injected subcutaneously into each of two rabbits. Rabbit no 1 received inoculation with vaccinia virus (B33) by scarification immediately afterwards and rabbit no. 2 24 hours later As controls both rabbits received by injection at a different site 0 5 ml of a solution of 0 03 tricresol in water These control sites were

Table VII The effect of the administration of material IV (from ox blood) to children prior to or immediately after the inoculation of vaccinia virus (cow pox vaccine). Six children were given in the left arm intracutaneously 0.1 ml (0.48 mg) of sterile solution of material IV. Three of the children received immediately after inoculation with vaccine on the same site and the remaining three children 24 hours later. All children were inoculated with vaccine in the right arm. The results were recorded 8 days later

Child no.	Time (hours) between injection of material and inoculation	Reaction on right arm	Reaction on left arm
1	0	+	weakly +
2	0	+	—
3	0	+	—
4	24	+	—
5	24	+	weakly +
6	24	+	—

+ = positive.
— = negative.

inoculated immediately afterwards with vaccinia virus by scarification. The reactions were read 6 days later. Where material IV had been injected, there was a 0.5 cm × 0.5 cm area free of lesions in rabbit no. 1 and in rabbit no. 2 the area was 1 cm × 0.5 cm. In the controls, however there were no areas which were free of lesions.

Material prepared from human blood has also been tested. 0.5 ml of a solution of the material (0.08 mg/ml) was injected intracutaneously into each of two rabbits. Two hours later rabbit no. 1 was inoculated by scarification with vaccinia virus (T)166, diluted 10^{-3}) and 26 hours later rabbit no. 2. After 6 days rabbit no. 1 had a 2 cm × 1 cm area free of lesions and in the second rabbit the area was 1.5 cm × 1 cm.

Table VIII The protective effect of the materials prepared from liver and blood on E. coli infection in mice. In each of the lines of experiments recorded a control group of six mice receiving bacterial challenge only was used. In no case was there any survivors in the control groups. The groups of experiments in which the same number of bacteria were used, are not strictly comparable, since the virulence varied somewhat from experiment to experiment

Material injected prior to bacterical challenge	Amount of material injected mg	Number of organisms injected	Hours between injection and bacterial challenge	
			3	6
			Survivors/total mice	
Material II	0.2	1.25 10^{-}[illegible]	1/6	3/6
Material II	0.2	1.25 10^{-}[illegible]	2/6	6/6
Material II	0.2	2.5 10^{-}[illegible]	2/6	0/6
Material II	0.2	2.5 10^{-}[illegible]	4/6	3/6
Material III	0.2	1.25 10^{-}[illegible]	4/6	2/6
Material III	0.2	2.5 10^{-}[illegible]	1/6	1/6
Material IV	0.096	1.25 10^{-}[illegible]	4/6	5/6

Injected intramuscularly in saline.
Injected intraperitoneally in saline.
Injected intraperitoneally in saline containing 0.3 % tricresol (control experiments showed that there was no effect of tricresol).

THE EFFECT OF MATERIAL I AND IV ON INOCULATION OF CHILDREN WITH COW POX VACCINE

In table VI and VII experiments are described which were designed to study the protective effect in man. It appears (table VI) that 12.5 mg of material I (from liver) abolished completely the local effect of the inoculation of the virus when the material was injected 24 hours prior to inoculation. When the material was injected simultaneously with the virus, a definite effect was also observed. Furthermore it is seen (table VII) that 0.48 mg of material IV (from blood) abolished completely the effect of the

intestine inhibits the infectivity of GD VII strain of Theiler s encephalomye litis virus (9)

A protein or protein complex which tolerates 50—60° is present in sera from horses and rabbits (10) It neutralizes the infectivity of Asian influenza viruses. These inhibitors have not been demonstrated in sera from humans or other susceptible hosts and their role in resistance to infection is therefore uncertain.

A sulphated polysaccharide present in agar which is inhibitory in a wild type of encephalo-myocarditis virus (11) has also been described. Furthermore it has been shown (12) that endotoxins protect mice against challenge with horse-encephalomyelitis virus, encephalomyocarditis virus and against influenza virus. The endotoxins are bacterial lipopolysaccharides.

The materials described from liver and blood interferes with the growth of the vaccinia virus in chick embryo in surprisingly small doses ($< 5\ \mu g$ per embryo) Present analytical data (part I) on the materials therefore tend to support the belief that the active substance is a polypeptide. In this respect it resembles interferon (6) The active materials described in this paper however seem to be more heat stable (tolerates 100°) than interferon. An interferon appearing in a cell culture infected with measles virus (7) retained activity after 2 hours at 56

Like interferon (12 13) when injected intradermally int rabbits, it protects against the development of lesions by the vaccinia virus inoculated in the same site

When chick chorio-allantoic membranes are incubated with interferon in a fluid medium, the growth of a challenging virus will be suppressed. But interferon does not act when inoculated on the chorio-allantoic membrane or injected into the allantoic cavity (12) of chick embryos challenged with vaccinia. In this respect there is a difference between interferon and the materials described in this paper since the latter are active both in the skin test and in the live chick embryo.

Material I (from liver) prevents the development of vaccinia lesions in children (table VI) This is interesting since t shows that the virus inhibitor in our material is effective in man. This has not yet been shown to be the case with interferon. In this connection t is worth mentioning that a large scale clinical trial on soldiers (double blind test) carried out 2 years ago showed that upon intramuscular injection of material I some protection against acute respiratory infections was achieved (1)

The materials prepared from liver and blood suppressing the growth of vaccinia virus also increase the resistance of mice to E. coli infection. Whether interferon possesses this property is not known. The property of protecting mice against E. coli infection is one which is usually associated with bacterial lipopolysaccharides (4) These substances are antigenic and pyrogenic. The materials described in the present paper do not seem to be pyrogenic when tested in rabbits n a dose considerably higher than that used in the mice. Material II (from liver) and material IV (from blood) were not found to be antigenic with the test employed. The presence of any significant quantities of bacterial lipopolysaccharides is not therefore considered very likely Bacterial lipopolysaccharides also stimulate the reticuloendothelial system. Our materials prepared from liver do not have this property (14)

Table IV Temperature increases in rabbits upon injection of materials from liver and blood

Material no.	Amount injected mg[1]	Maximum increase in body temperature of rabbit no.							
		1	2	3	4	5	6	7	8
I	125	0.5	0.3	0.6	0	0.5	0.8	0.4	0
II	2.06	0.5	0.1	0	0.1	—	—	—	—
III	3.6	0.5	0.5	0.6	0.7	—	—	—	—
IV	4.8	0.3	0.3	0.4	0.5	—	—	—	—

Dissolved in pyrogen free water containing 0.1 ml of tricresol. Upon injection of a similar volume of the same water containing 0.3 of tricresol only the following increases in temperatures were found 0.2 0.3 0.4 and 0.4

Table V Testing of material II (from liver) and IV (from blood) on antigenicity 1 ml of a solution of the material (5 mg/ml and 4 mg/ml in the case of material II and IV respectively) in water containing 0.3% tricresol were injected into each of three normal human volunteers. The solutions injected subcutaneously had the same concentration of material. The area of infiltration and rubor is given in mm

Volunteer no.	Histamine 0.1		Undiluted material		Diluted material	
	Infiltration	Rubor	Infiltration	Rubor	Infiltration	Rubor
	Material from liver					
1	10 × 12	40 × 45	4 × 4	15 × 20	0	0
2	11 × 11	35 × 35	0	10 × 15	0	0
3	15 × 16	40 × 40	4 × 4	0	4 × 4	0
	Material from blood					
1	10 × 10	50 × 55	5 × 5	5 × 5	5 × 5	5 × 5
2	10 × 12	30 × 40	6 × 6	6 × 6	5 × 5	6 × 6
3	12 × 2	35 × 40	6 × 6	6 × 6	6 × 6	6 × 6

Diluted 1 10,000.
Diluted 1 1,000.

vaccine in four out of the six cases In the remaining two children there was a definite effect although less pronounced

THE PROTECTIVE EFFECT OF MATERIALS PREPARED FROM LIVER AND BLOOD ON E. COLI INFECTION IN MICE

It is seen from table VIII that materials prepared from both liver and blood produce some protection against E. coli challenge in mice

PYROGENICITY AND ANTIGENICITY OF MATERIALS

The temperature increases in rabbits following injection of materials prepared from liver and blood are seen in table IV. The doses given are considerably higher than those used in the experiments previously described and none of the materials are considered to be pyrogenic

It is seen in table V that material II and IV respectively are not antigenic in man

Discussion

The substance(s) present in the materials prepared from liver and blood responsible for the antiviral activity against vaccinia is of a high molecular weight (see part I) Several substances of high molecular weight exhibiting antiviral activity have recently been described The most prominent being, a substance called interferon, first described by Isaacs and Lindenmann (5) which is a protein with a molecular weight of about 63 000 (6) It is produced as a result of infection of cells with several strains of influenza virus, measles (7) poliovirus, vaccinia virus, vesicular stomatitis virus and polyoma virus (5)

A number of serum and tissue inhibitors of virus has been reported (8) Some of these are of high molecular weight and inhibit virus infectivity For instance a mucopolysaccharide obtained from the wall of the mouse stomach and small

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Life and Death without Ovaries

By

Erik Ask Upmark

The present investigation was performed in order to find out whether the morbidity and the mortality of women, deprived of their ovaries, did essentially differ from the normal. There were at the onset two questions in particular to be answered.

1. Will a bilateral oophorectomy influence the occurrence of malignancy? It is well known that male eunuchs although at a disadvantage in so many regards, rarely if ever get prostatic carcinoma. On the other hand, the favourable influence of estrogenic hormones in many cases of prostatic carcinoma with metastases is a well-established fact. As to women, it has been maintained that mammary carcinomas might be favourably influenced by castration of the patient.

2. Will a bilateral oophorectomy bring about any increased disability with regard to the cardiovascular system? The well known predilection of coronary infarctions for males has been explained differently. On the one hand it has been maintained, rightly in my opinion, that the anatomy of the coronary arteries is different in men and women, the intima being considerably thicker in males, even so when new-born. It is possible that this feature is essential. On the other hand the *presumption* has been made that the male preponderance might be due to some endocrine factor where particularly the estrogenic hormone should be about. This presumption, which has to some degree been substantiated by animal experiments, has even induced some authors to suggest the use of estrogen preparations in males, in order to reduce the risk of coronary infarctions. A few papers with bearing on this topic have already appeared.

Material

The material was represented by women, who had been subjected to bilateral oophorectomy in the department of obstetrics and gynecology of Upsala. For the kind permission to use this material I am obliged to my colleagues in this department, Professor John Naeslund (now retired) and Professor Carl Axel Gemzell (now in charge).

The patients concerned were operated upon in the decade 1920—1930. The follow-up period was accordingly long and another

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The substance(s) present in the materials prepared from liver and blood could have something to do with the body's defence system against viral and possibly bacterial challenge and is perhaps present in any mammalian cell They could be part of the system of "natural immunity" (8)

The purified material from blood and from liver exhibits a green yellow fluorescence (part I) in ultraviolet light If the fluorescent substance is a constituent of the biological active substance, it might be possible to measure the degree of "natural immunity" by the intensity of the fluorescent material present in blood.

Recently Raskova and Vanecek (15) found that repeated injections of phenol *increase the resistance of mice against* toxins and traumatic shock. It is suggested that the phenol stress brings about a release of a resistance increasing substance in the blood, since it has been shown that increased resistance can be passively transferred to nontreated animals Thus resistance factor could possibly have something to do with the active substance in the materials described in the present paper It is interesting to note that these materials seem to protect mice against a cold stress (16)

Summary

Materials prepared from mammalian liver and blood interfere with the growth of vaccinia virus in chick embryo rabbit's skin and man The materials increase the resistance of mice to E. coli challenge and have been shown to be neither pyrogenic nor antigenic.

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Finally it is a great pleasure to thank Dr H. M. Walker O. B. E. of the Glaxo Group for encouragement and many valuable discussions.

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Comments (cf. table II)

I. SURVIVORS

Biliary disorders As such were considered cholelithiasis or/and cholecystitis, as a rule substantiated by operation. These disorders were equally common in both groups (bilateral and unilateral oophorectomies) and concerned about one case out of 4 In most instances these biliary disorders occurred several years (16—37 years) after the gynecological operation.

Cardiac disorders In the survivors after bilateral oophorectomy a certain degree of dyspnoea on effort or even angina pectoris on effort was noted during the last years in 10 cases, not very remarkable if their age is considered. In one case a paroxysmal tachycardia was present and only in one case a coronary infarction, 28 years after the operation. In the survivors after unilateral oophorectomy heart troubles were more common hypertension was said to be present in 8, dyspnoea on effort during the last years in 5 vascular cramp in 11 and coronary infarction in one woman, 30 years after the operation.

Endocrine disorders In the "bilateral group one case had, when followed up Addison s disease with calcification of the suprarenals, on case developed a thyrotoxicosis 14 years after the gynecological intervention, 4 cases developed indifferent goiters 13—31 years after the operation, 1 case striking obesity limited to the abdominal and pelvic region during the year immediately after the operation and in t least one case the deficiency symptoms were judged to be severe. This woman was only 15 when operated upon and she still shudders, 31 years later when remembering the ordeal of her first postoperative years I had pains in my abdomen every day I was restless as a ghost and could not sleep at all any night during the first year after the oper ation, I was extremely nervous and my heart rate accelerated, my pulses throbbing they should not be allowed to do such things to a young girl" — As to the unilateral group" there were, among the survivors two cases of in different goiter one case of thyrotoxicosis and one case of diabetes.

Joint disorders In the questionary a special line was devoted to the joints since we have earlier repeatedly observed the appearance of severe rheumatoid arthritis in connection with the menopause, particularly if uterine bleeding during that time has been checked by X-ray treatment. The results of the present investigation were meagre in this regard. Among the bilateral oophorectomies only 4 complained of symptoms from their joints in one transitory symptoms immediately after the operation, in one, who had rheumatoid arthritis already before the operation, th joint disease was markedly accelerated after the oophorectomy and in two instances a rheumatoid arthritis developed several years after the intervention. As for the control group as many as 8 had had difficulties with their joints in one the arthropathy appeared immediately after the operation, in the rest rheumatoid disorders appeared later in lif particularly at the age of the menopause.

II. DECEASED

Cardiac disorders In 11 out of the 38 women who have died deprived of both ovaries the cause of death was cardiac (21—37 years after the operation) allegedly valvular failures in 2 and cardiosclerosis in 9 (coronary thrombosis is mentioned once and cardiac infarction

Table I Oophorectomies in women 15—45 years

	Bilateral	Unilateral
Alive at follow-up	65	77
Dead when followed-up	38	20
Total	103	97
	(years)	(years)
Average age operation	36.7	33
Average follow-up		
Cases alive	34	33
Cases dead	16.4	16

Table II Disorders of the 200 patients of table I

	Bilateral	Unilateral
Survivors when followed-up	65	77
Biliary disorders	15	18
Cardiac disorders	12	28
Endocrine disorders	8	4
Joint diseases	4	8
Dead when followed-up	38	20
Cardiac death	11	5
Cerebrovascular death	5	0
Neoplasm	11	5
Suicides	2	1
Diabetes	2	1
Various causes	7	8

advantage, from the point of view of the investigation, was that several of these patients had died during the years gone by and that their causes of death accordingly could be registered. It may also be presumed that, at least in the majority of this material, no substitution therapy has been used since it was scarcely available in the twenties. On the other hand certain disadvantages with such a long follow up period should not be denied. Firstly it turned out to be impossible to trace several of the cases and this admittedly reduces the value of the follow-up. Secondly although strikingly many of the patients had obviously preserved their mental abilities, a few of the old ladies were hardly in any too good cerebral condition and their memory of disorders in the past was to some extent clouded by the years. A certain compensation in this regard was obtained by the kind information yielded by doctors or nurses in charge of the old people's home where several of these women had eventually arrived. I am obliged to these medical assistants, to the gentlemen of the clergy and to the central registration office of the population of the province for valuable assistance in tracing the patients. Last but no least I acknowledge with gratitude the willingness and kindness of the patients themselves.

The patients were in most instances operated upon because of myoma of the uterus although other disorders were included as well. As control material women subjected to one-sided oophorectomy during the same period were used. If the patients were dead at the follow up (undertaken mainly in May 1960) the cause of death was recorded and if they had died in a hospital additional information was obtained from the department concerned (not a few of them had died in my own department of medicine). If the patients were alive at the time of the follow-up a questionary was sent to them to be filled in. This questionary was intentionally confined to essential things: whether they had been all right since the operation or not, whether, if not, they had been subjected to other hospital periods (when and where and for what), whether they had been operated upon for any tumour (when, where) or whether they had had any disturbances of the heart or the joints, or whether any other disease had been about. They were told that if they wanted to appear themselves they were welcome, understanding that the examination would cost them nothing. Since however many of these women were from rural districts it had been necessary to meet their travelling expenses if all of them had come. We had no funds for this purpose and accordingly the majority of the survivors were examined by the questionary only, which of course is to be regretted.

The material of oophorectomies was divided into those operated upon when 15—45 years of age and into those operated upon later. Table I gives a survey of the material.

suicide in both instances 12 years later) In the control group one woman, aged 33 when operated upon, committed suicide 10 years later (submersion)

Diabetes was indicated as the cause of death in 2 women, subjected to bilateral and in 1 woman subjected to unilateral oophorectomy

As for the women more than 45 years of age when operated upon tables III—V may summarize the observations.

Discussion

It is obvious that in addition to the objections already mentioned the present material is of much too limited size to allow any definite conclusions. We have outlined an investigation on a larger scale in collaboration with the departments of obstetrics and gynecology in Upsala and in Lund Malmö The present study may be considered as a preliminary reconnoitring of the field implied.

With regard to suicides, endocrine disorders and joint diseases no conclusive differences were to be elicited between the material subjected to bilateral oophorectomy and the controls. It is possible that a larger material may yield divergent evidence on this matter

Diabetes was found in 5 out of 228 women deprived of both ovaries, in 2 out of 126 women who had one ovary removed, rather normal incidence in both cases.

Biliary disorders were equally common in both materials t seems instructive that at least one woman out of four in this material has clinical symptoms from her biliary tract.

As to cardiovascular disorders suggestive evidence has been published by Pick et al (4) They performed ovariectomy in hens, with resultant loss of resistance to cholesterol feeding In women reports have been published by Wuest et al. (7) and by Rivini and Dimitroff (5) on the pathological anatomy of the coronary arteries. Wuest at the Mayo compared 49 hearts from women subjected to bilateral oophorectomy with observations previously published on the coronary pathology in women (Ackerman, 600 women) and men (White, 600 men) He considered the coronary arteries to be more severely involved, decade for decade, in the oophorectomy-material when compared with the female controls although not as severely as in the male controls. Rivini in Los Angeles made a similar study in 90 women, castrated before the age of 50 and surviving at least one year their coronary alterations exceeded those of the controls (5) On the other hand it was stated that males with cancer of the prostate, subjected to stilbol treatment presented less pronounced coronary affections at the post mortem than did ordinary men. Similar results were obtained by London et al. (2) in New York (post mortems of coronary arteries in males with carcinoma of the prostate, treated and untreated with estrogens) Clinical reports have been published by Robinson et al. (6) from Worcester Mass., and by Oliver and Boyd (3) from Edinburgh. Robinson et al. found coronary symptoms in 16 out of 102 women, castrated prior to the menopause, but only in 5 out of 112 controls (cases obtaining substitution treatment were excluded) Oliver and Boyd made a thorough examination, including electrocardiography and cholesterol determinations in 36 bilateral and 31 unilateral oophorectomy patients. Among the former there were 27 with cardiac disturbances (one infarction) among the controls there were 10 (no

Table III Oophorectomies in women older than 45 years

	Bi-lateral	Uni-lateral
Alive at follow-up	19	5
Dead when followed-up	106	24
Total	125	29
Average age, operation	(years)	(years)
Survivors	50.3	53
Later deceased	54.2	59.2
Average follow-up		
Survivors	33.9	27
Deceased	18	16.1

Table II Destinies of the 154 patients reproduced in table III

	Bi-lateral	Uni lateral
Survivors	19	5
Biliary disorders	6	2
Cardiac disorders	6	—
Endocrine disorders	1 (diabetes)	—
Deaths	106	24
Cardiac	25	5
Cerebrovascular	23	2
Neoplasms	31	6
Suicide	1	0

In 3 instances valvular failures, in 22 cardiosclerosis (infarction is mentioned in 3).

In 2 cases cardiac insufficiency in 1 coronary arteriosclerosis"

The difference is striking and likely to be quite real.

In 15 cases digest ve tract (stomach, colon, pancreas, liver). In 5 cases pelvic organs (uterus mostly). In 2 cases cancer mammae. In 3 cases various other organs, in one case sarcoma (no localization) in the rest only "cancer"

In 2 cases digestive tract (stomach, colon) in 2 cases pelvic organs (uterus once, sarcoma ovarii once) In 1 case cancer mammae. In 1 case "cancer"

Table V Oophorectomy in women 1920—1930 All cases grouped together irrespective of age at the oophorectomy

	Bi-lateral	Uni-lateral
Alive when followed-up	84	82
Deceased when followed-up	144	44
Total	228	126
Cardiac deaths	36	8
Cerebrovascular deaths	28	2
Neoplastic deaths	42	11
Suicides	3	1
Cardiac disorders during life	18	23

once) As for the control group 5 had died a cardiac death out of 20 in 3 instances a valvular lesion is said to have been present in 2 cases a cardiac infarction (in one of these diabetes was present as well)

Cerebrovascular deaths were noted in 5 instances of bilateral oophorectomies (11—31 years after the operation) and in no case in the control group

Neoplasm as cause of death was recorded in 11 of the bilateral and in 5 of the unilateral operations. The 11 tumours in the bilateral group" were localized to the digestive tract in 4 instances, to the pelvic organs in 3 to the brain in 2 and only as "cancer in 2 As for the control group there was one case in each of the following localizations collum uteri, mammae uterus, gall-bladder and "cancer [1]

Suicides were reported in two women subjected to bilateral oophorectomy (when operated 40 resp 45 years of age,

[1] It should be observed that 2 more women of the control group have been operated for carcinoma mammae but survived so far (13 and 3 years ago)

suicide in both instances 12 years later). In the control group one woman, aged 33 when operated upon, committed suicide 10 years later (submersion).

Diabetes was indicated as the cause of death in 2 women, subjected to bilateral and in 1 woman subjected to unilateral oophorectomy.

As for the women more than 45 years of age when operated upon tables III—V may summarize the observations.

Discussion

It is obvious that in addition to the objections already mentioned the present material is of much too limited size to allow any definite conclusions. We have outlined an investigation on a larger scale in collaboration with the departments of obstetrics and gynecology in Upsala and in Lund-Malmö. The present study may be considered as a preliminary reconnoitring of the field implied.

With regard to suicides, endocrine disorders and joint diseases no conclusive differences were to be elicited between the material subjected to bilateral oophorectomy and the controls. It is possible that a larger material may yield divergent evidence on this matter.

Diabetes was found in 5 out of 228 women deprived of both ovaries, in 2 out of 126 women who had one ovary removed: rather a normal incidence in both cases.

Biliary disorders were equally common in both materials: it seems instructive that at least one woman out of four in this material has clinical symptoms from her biliary tract.

As to cardiovascular disorders suggestive evidence has been published by Pick et al. (4). They performed ovariectomy in hens, with resultant loss of resistance to cholesterol feeding. In women reports have been published by Wuest et al. (7) and by Rivini and Dimitroff (5) on the pathological anatomy of the coronary arteries. Wuest at the Mayo compared 49 hearts from women subjected to bilateral oophorectomy with observations previously published on the coronary pathology in women (Ackerman, 600 women) and men (White, 600 men). He considered the coronary arteries to be more severely involved, decade for decade, in the oophorectomy material when compared with the female controls although not as severely as in the *male* controls. Rivini in Los Angeles made a similar study in 90 women castrated before the age of 50 and surviving at least one year: their coronary alterations exceeded those of the controls (5). On the other hand it was stated that males with cancer of the prostate, subjected to stilbol treatment presented less pronounced coronary affections at the post mortem than did ordinary men. Similar results were obtained by London et al. (2) in New York (post-mortems of coronary arteries in males with carcinoma of the prostate, treated and untreated with estrogens). Clinical reports have been published by Robinson et al. (6) from Worcester, Mass., and by Oliver and Boyd (3) from Edinburgh. Robinson et al. found coronary symptoms in 16 out of 102 women, castrated prior to the menopause, but only in 5 out of 112 controls (cases obtaining substitution treatment were excluded). Oliver and Boyd made a thorough examination, including electrocardiography and cholesterol determinations in 36 bilateral and 31 unilateral oophorectomy patients. Among the former there were 27 with cardiac disturbances (one infarction): among the controls there were 10 (no

infarction) The evidence hitherto assembled seems accordingly suggestive of some protective influence exerted by the ovaries on the coronary vessels

As for the present study a somewhat similar conclusion appears to be justified If for the sake of argument the deaths recorded in table V are chosen we get 31 cardiac deaths out of 144 women with bilateral oophorectomy (+ 5 cases of valvular failure) but only 5 such deaths out of 44 women with unilateral oophorectomy (+ 3 cases of valvular failure) Even if the small material recorded in table II is considered (women operated upon when 15—45) a similar difference is to be seen If the valvular failures are subtracted we get 9 deaths of reasonably coronary origin out of 38 (about one case out of 4) as against 2 such deaths among 20 deaths in the control group (one death out of 10) If women operated upon when more than 45 are considered (table IV) and the valvular failures likewise are subtracted we get 22 reasonable coronaries out of 106 deaths as against 3 out of 24 deaths among the controls The trend is thus fairly unanimous although a somewhat disturbing fact is the more common cardiac disturbances among the survivors after one-sided oophorectomy There is, however one piece of evidence perhaps the most conclusive of all in this study supporting the conception of an affection of the cardiovascular system as more common in castrated women the striking dominance of cerebrovascular deaths in the castrate as compared with the controls. Such deaths are generally due either to hypertension or to arteriosclerosis or to both. It cannot be maintained that the women in the castrated group were significantly older than the controls. Accordingly it seems as if the present study might furnish corroborative evidence to the conclusions suggested by the authors already quoted.

With regard to the occurrence of malignant tumours in castrated and in not-castrated women the present material is much too heterogeneous and of too limited size as to be able to furnish any definite conclusions. However if the occurrence of malignant tumours also in the survivors is considered there were among 228 bilaterally oophorectomized women only 2 carcinomas of the mammae (both dead) whereas out of 126 unilaterally oophorectomized women no less than 4 had got mammary carcinoma (two dead two survivors) It is obvious that the figures are much too small, yet a certain suggestion might be ventured that some degree of protection from mammary carcinoma possibly may be yielded by castration.

Summary and conclusions

1 Bilateral oophorectomy was carried out in Upsala 1920—1930 in 228 women, who have been followed up in 1960. Unilateral oophorectomy was performed at the same period in 126 women, who likewise have been traced and served as controls. There were several other cases where it has been impossible to trace the patients, a matter reducing the value of this investigation The present follow up will serve as a pilot study for a more detailed follow-up of a larger material in collaboration with colleagues in Upsala and Lund.

2 Biliary disorders were noted in 25 % of the survivors the frequency being the same in the castrates as in the controls.

3 No conclusive difference was present between the occurrence of suicides, endocrine disorders or joint affections in both materials.

4. Cardiac deaths, reasonably of coronary origin were more common in the group of castrates than in the control group. However among the survivors more cardiac complaints were made by the control patients than by the castrates.

5. Cerebrovascular deaths were much more common in the castrates (28 cases out of 144 deaths) than in the controls (2 cases out of 44 deaths)

6. Malignant tumours occurred about as often in the subsequent course of castrates as in the controls. However among 228 castrates only 2 cases of mammary carcinoma were noted, where as 4 such cases were to be found among the 126 controls. This point as well as the others needs further elucidation and so does the fact that not one case of leukemia was observed among 188 deaths (144 castrates 44 controls) in spite of the long follow-up.

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Complete Anomalous Drainage of Left Pulmonary Veins

By

J. Nieveen, Hr. de Vries and F. ten Hoor

In partial anomalous drainage of pulmonary veins, abnormalities involving the veins of the right lung are more common than those involving veins of the left (1). According to Edwards (2) from an embryological point of view there is no explanation for this preference. In this paper we present a case, in which all the veins of the left lung are draining into the right side of the heart. We want to publish the data about this patient and to stress once more the fact, that minor physical, electrocardiographic and radiological signs do not exclude major abnormalities.

Case report

The patient, a 37-year-old man, had attacks of dyspnoea since early childhood. Some weeks before admission to hospital the dyspnoea became more severe. H. had no complaints between the attacks.

On examination we found a normally built man with slight kyphoscoliosis, slight cyanosis and moderate dyspnoea. The blood pressure was 130/90. The pulse was regular with a frequency of 90 per minute. On auscultation of the lungs a prolonged expiratory phase with diffuse wheezing rhonchi in both lungs was found. Over the heart in the fifth intercostal space at the apex, a very soft systolic murmur was heard, which disappeared during inspiration.

The sputum contained 90 % eosinophilic cells. Respiratory functional studies revealed a vital capacity of 3,890 ml (normal value for this patient 5,000 ml). The timed vital capacity was 24 % in 1 second (calculated value 69 %). Arterial oxygen saturation 88 % (on admission).

The radiological examination (fig. 1) showed a bilateral depressed diaphragm with adhesions on the left side. The cardiac silhouette was slightly displaced to the left; the pulmonary artery segment was somewhat enlarged; with fluoroscopy a slight hilar dance was seen.

Electrocardiographically (fig. 2) there was a normal sinus rhythm and no signs of right or left hypertrophy. The phonocardiogram (fig. 2) confirmed the auscultatory findings.

Cardiac catheterization was performed in order to study the pressure in the pulmonary artery both during basal conditions and after exercise. The data obtained are presented in table I. Pressures were measured by means of a Statham P23Db pressure transducer recording on a Sanborn Polyviso 150 M with a carrier preamplifier. The oxygen saturation values were obtained by direct oximetry with a reflection oximeter (CC oxymeter, Kipp and Sons, Delft, Holland [7]). Note the remarkably high oxygen saturation values in

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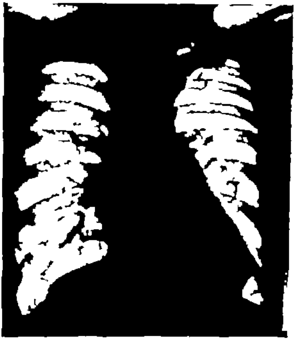


Fig 1

Table I Pressure and oxygen saturation data during cardiac catheterization

Place of catheter tip	Pressure (mm/Hg)	Oxygen sat. %
Right jugular vein	—	65,67
Superior vena cava (near RJV)	m.p. 4	72,74
Superior vena cava (second intercostal space)	—	86,87
Superior vena cava (third intercostal space	m.p. 4	86,87
Right atrium (high)	—	83 84
Right atrium (mid)	m.p. 5	82,84
Inferior vena cava (near RA)	—	78
Hepatic vein	—	67
Inferior vena cava (low)	—	85
Right ventricle	35/0	85,86
Main pulmonary artery	35/10	85
Left pulmonary artery (wedge)	m.p. 12	—
Left pulmonary artery	—	81
Right pulmonary artery	—	81,83,84
Left atrium (via ASD)	—	99
Brachial artery	—	96,97

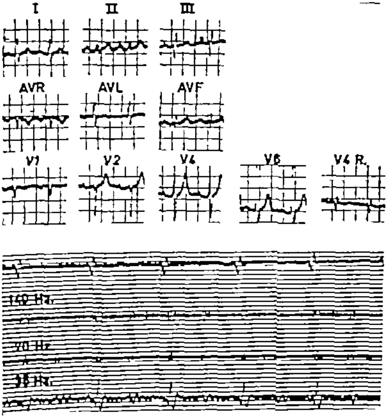


Fig 2.

the superior vena cava, while in the right jugular vein and the inferior vena cava oxygen saturation is normal.

Dye dilution curves were routinely made, using Fox green as the dye (fig 3). The curves were obtained by sucking blood from the brachial artery through a cuvette, in which the dye concentration is measured by reflectometry in infrared light. A pump unit maintains constant flowrate of 0.80 ml/sec. in the sampling system (5—6). Recorder Sanborn polyviso 150 M with low level preamplifier. Paper speed 5 mm/sec. The first three curves show a small early concentration peak before the normal one and an abnormal recirculation peak after the normal one. The fourth curve shows a small abnormal recirculation peak after the normal concentration peak (8). Note the significant difference in appearance time between the first two curves and the third one.

Because of the abnormal findings of these curves, we decided to do selective angiocardiography (Prof. J. R. Blickman, M. D. Radiological Institute, University hospital, Groningen). Two of the radiographs are shown in figures 4 and 5.

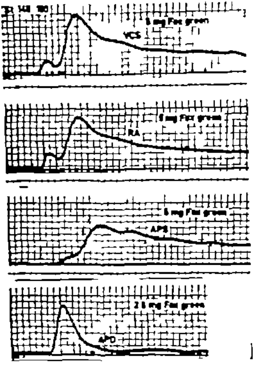


Fig. 3. Dye dilution curves. Injection sites superior vena cava (VCS), right atrium (RA), left pulmonary artery (APS), right pulmonary artery (APD).

Discussion

Although a soft systolic murmur was present at the apex and there were small X ray abnormalities, we did not primarily think of any cardiac abnormality.

During catheterization our attention was drawn to the remarkably high oxygen saturation in the superior vena cava. Compared with the oxygen saturation values in the right jugular vein and the inferior vena cava, it was clear that quite a large amount of highly saturated blood entered the venous system.

We also succeeded in reaching the left atrium, which in a 37-year-old patient is suggestive of an atrial septal defect.

With the aid of dye dilution curves it was possible to make a final diagnosis (fig. 3).

The early concentration peak, which is present when dye is injected into the superior vena cava and the right atrium, proves the presence of a small right-to-left shunt. However, when dye is injected into the left pulmonary artery, the curve also shows an early concentration peak, but the difference from the other curves is the significantly longer appearance time. For this curve only one explanation is possible: all the dye injected into the left pulmonary artery flows to the right side of the heart resulting in a curve with the same pattern as if dye were injected into the superior vena cava. Dye injected into the right pulmonary artery travels along the normal route, resulting in a curve with a normal concentration

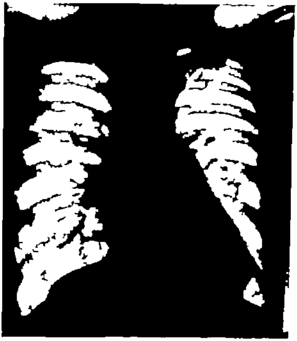


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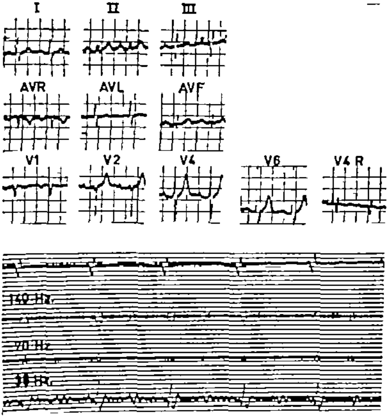


Fig 2

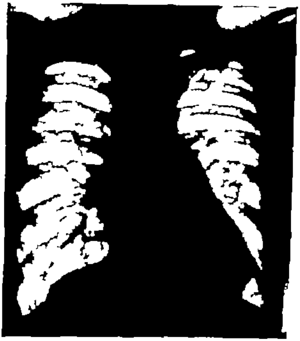


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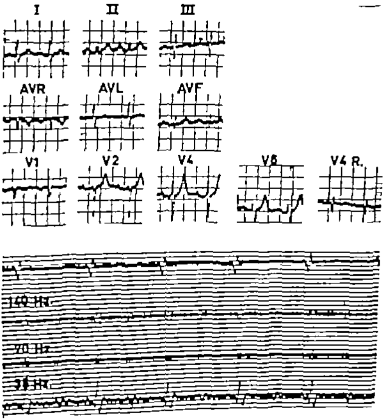


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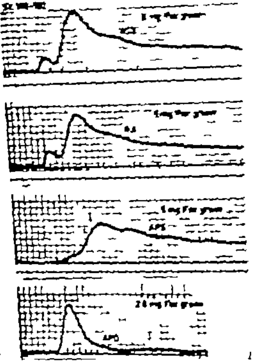


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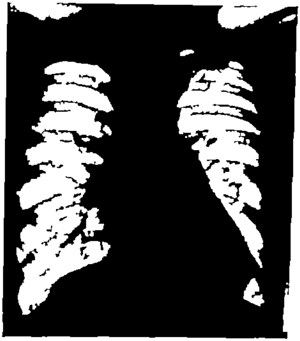


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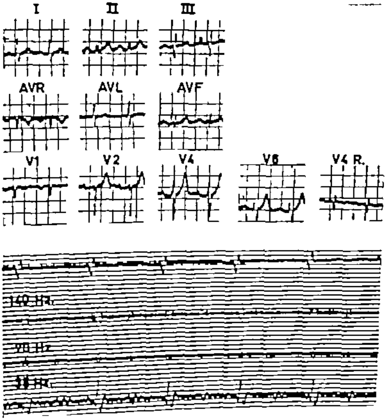


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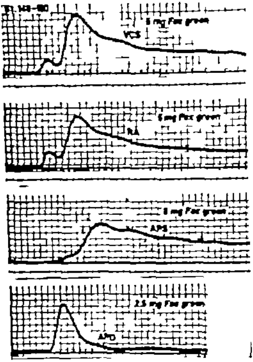


Fig 3. Dye dilution curves. Injection sites superior vena cava (VCS) right atrium (RA), left pulmonary artery (APS) right pulmonary artery (APD)

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Fig 4 Angiocardiogram 2 sec. after injection of 30 ml urografin into main pulmonary artery Injection was done with a Gidlund syringe. Pressure 3 atm.



Fig 5 Angiocardiogram 4 sec. after injection of 30 ml urografin into main pulmonary artery

peak followed by a small abnormal concentration peak which is caused by a small left to right shunt through the atrial septal defect.

From oxygen saturation values and dye dilution curves it was clear that the blood of the entire left lung was emptied high into the venous system and that an atrial septal defect was present.

Selective angiocardiography confirmed the conclusion about the complete abnormal drainage of left pulmonary veins and showed (fig 4 and 5) how these veins joined to form one large vessel, which emptied into the left innominate vein.

Although the bronchial asthma was not expected to be influenced by the described abnormalities, it was thought that an operation with closure of the atrial septal defect and transplantation of the abnormally draining vessel to the left auricle was indicated for two reasons

1 The pulmonary flow is greatly increased and will possibly damage the pulmonary vessels and the right heart.

2 Disease of the lungs, which in this patient with bronchial asthma is apt

to occur can become dangerous if the right lung is involved, as is clearly shown in a case report of Conant and Kurland (9)

For personal reasons the patient refused operation and was discharged from hospital after treatment of his bronchial asthma. So we did not succeed in establishing our diagnosis anatomically or correcting the abnormality

Summary

Complete abnormal drainage of left pulmonary veins was diagnosed in a 37-year-old man with bronchial asthma by means of cardiac catheterization and routinely made dye dilution curves. Selective angiocardiography showed the left pulmonary veins joining into one large vessel which empties into the left innominate vein.

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Addendum

After completing this report the patient died suddenly during an asthmatic attack. Post mortem findings established our diagnosis in all aspects (Prof. A. Arends, M. D.) The death during an asthmatic attack, very uncommon in this time, must be explained from the fact, that the patient had only one effective functioning lung. So the case is comparable to that of Conant and Kurland (9)

Publications received

Redaktionen sänder på anmodan böcker för recension

Beschreibende und funktionelle Anatomie des Menschen 3 Aufl Von Kurt Tittel 562 S Preis DM 30 — VEB Gustav Fischer Verlag Jena 1962

The Human Amnion and Chorion. By Gordon L. Bourne 276 pp 134 figs Price 45s. net Lloyd Luke Ltd London 1962

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Influence of Enzyme Therapy on Serum Cholesterol and Lipoprotein Values in Male Patients with Coronary Artery Disease

By

T. PETTERSSON and H. ADLERCREUTZ

The etiology and pathogenesis of atheromatosis and atherosclerosis are still not known but there is much evidence for the existence of a correlation between the occurrence of coronary sclerosis and the concentration, composition and colloido-chemical state of the lipids in serum. A modern approach is offered by the suggestion that lack of one or several enzymes might be the pathogenetic factor in atheromatosis. On this basis an enzyme preparation called Vasolastine (Manufacturer Fa. Polypharm, Rotterdam W Holland) has been developed. It is composed of citrogenase-lipoxidase, amino-acid oxidase and tyrosinase-tryptase. The first mentioned enzyme complex should exert a decomposing effect on atheromatous deposits and prevent the occurrence of new ones. The other two enzymes are intended to decompose amines effecting the blood pressure and the vascular wall.

In experiments on animals Hoogerwerf (1955) and Pettersson and Hjelmman (1960) noticed a favourable influence of this enzyme preparation on severe atheromatosis. Schulze (1958) could show a decrease of the serum total lipids 24 hours after administration of Vasolastine in about half the human subjects with a normal serum lipid concentration, whereas all subjects with an elevated serum lipid concentration exhibited a decrease.

The aim of this investigation was to study the influence of longterm enzyme therapy on serum cholesterol and lipoproteins in male patients with severe coronary disease.

Material and methods

The enzyme preparation was tested on 14 male patients, 12 of whom had been treated for cardiac infarction and two for stenocardia. Their ages varied between 26 and 60 years. The diagnosis of coronary thrombosis was made on the basis of previous histories, ECG-changes, leukocytosis and increase in the sedi-

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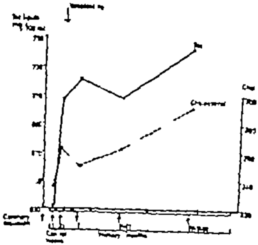


Fig. 1. Changes in the total cholesterol and total lipid concentrations in the serum of 14 patients during the time of observation.

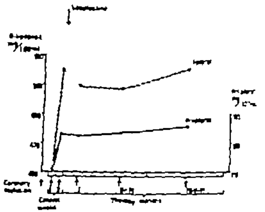


Fig. 2. Changes in α- and β-lipoprotein concentrations in the serum of 14 patients during the time of observation.

mentation rate as well as glutamic-oxaloacetic transaminase (GOT). The diagnosis of stenocardia was made on the basis of typical histories, ECG changes and exclusion of other diseases that might possibly have provoked the symptoms.

The period of hospitalization varied between 4 and 6 weeks. After that, the patients were treated at the outpatients department for periods ranging from 2 to 8 months, 5 months on the average. During the whole time of observation all patients were allowed an unrestricted diet. Nine patients were submitted to heparin therapy for the first 24 hours and after that to anticoagulant therapy with Marcoumar. The others received Marcoumar from the very beginning and this therapy has been continued for all patients. The Vasolastine therapy was initiated after three weeks hospitalization, the two last weeks serving as control weeks. During the two first weeks of therapy 1 ml Vasolastine was administered intramuscularly twice a week and after that 2 ml a week. In the meantime determinations of the serum cholesterol concentration were made once or twice a week and after that about once a month, according to the method described by Pearson, Stern and McGawack (1953). The lipoproteins were simultaneously investigated by paper electrophoresis as recommended by Grassmann and Hannig (1950). The specimens were always taken in the morning at the same time, the patient having fasted overnight.

Results

The blood cholesterol decreased in seven subjects but in three of them only one value lower than the starting value was noted after the initiation of therapy. In four cases the cholesterol values were unchanged, and in three cases a slight increase could be observed. The cholesterol values were subjected to statistical analysis according to the principle of analysis of variance and the result appears from fig. 1. The cholesterol values decreased significantly ($p < 0.001$) but temporarily during the first month of therapy. The value at the end of the period of therapy is even somewhat higher than the initial value.

The values for total lipids, α- and β-lipoproteins were also treated statistically in the same way and the results are shown in figs. 1 and 2. The total lipids decreased in the course of the second to third month of therapy, but this was also a temporary change. The decrease was less significant ($p < 0.005$) than the decrease for cholesterol. For the α- and β-lipoproteins no statistically significant change could be demonstrated in this material during the treatment although there was

a tendency towards a temporary decrease of the β-lipoproteins.

Most of the patients improved subjectively during the therapy Only one had a recurrence. The patient had then discontinued the therapy on his own initiative, but in connection with the recurrence of infarction it was resumed All patients are alive more than a year after the initiation of therapy No secondary effects of the preparation have been noticed with the dosage employed.

Discussion

Several investigations (Welin 1948, Biörck, Blomqvist and Sievers 1957 Miettinen 1957 Dodds and Mills 1959) show that in the first week after cardiac infarction the cholesterol and lipoprotein values decline only to rise later to the initial value or still higher These extensive studies can be compared with our investigation. Their findings concerning the three first weeks after the infarction agree closely with our values for the control weeks. When our results for the subsequent weeks are compared with those of Dodds and Mills the only difference is found to be that in our study the rise in the cholesterol and lipoprotein values during the second and third week after the infarction was arrested when Vasolastine therapy was commenced in the fourth week. This effect can hardly be due t anything else than the enzyme therapy

Throughout the period of therapy the subjective health of the patients was improved. Obviously this may be due to many causes other than the effect of the drug The regular medical attendance and the suggestive effect which accompanies a regular course of injections will certainly greatly affect the patient's well-being It is therefore very difficult to say whether or not any importance should be attached to the apparently favourable effect on the subjective symptoms. It should be remembered that all patients also received anticoagulant therapy

It is evident that the influence of Vasolastine on the lipid values of the blood does not in any case persist for more than one or two months, and therefore no definite conclusions concerning the usefulness of the preparation can be drawn.

Summary

Fourteen male patients with coronary artery disease have been treated with the enzyme preparation Vasolastine (Fa. Polypharm, Rotterdam W., Holland) for an average period of 5 months and the cholesterol, total lipid and α- and β-lipoprotein concentrations of the serum have been studied.

The cholesterol and total lipid concentration of the serum decreased significantly but temporarily during the first to second month of therapy whereas the lipoproteins did not show any significant changes.

The patients subjective well-being clearly improved during therapy but whether this was attributable to the enzyme therapy cannot be stated.

Vasolastine therapy in the doses given did not produce any secondary side effects.

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The Plasma Disappearance of Intravenous Cobalt-57 Vitamin B_{12} in Vitamin B_{12}-Deficiencies

By

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One would expect that the vitamin B_{12} deficient tissues of pernicious anemia would remove an intravenous dose of vitamin B_{12} at a faster than normal rate. Surprisingly three groups of investigators (1, 2, 3) found the plasma disappearance to be delayed. Brody et al. (3) found that the abnormality persisted, but to a lesser degree, in patients who had been treated. They also found 4 of 5 patients with steatorrhea and low plasma B_{12} levels to have a delayed disappearance. However Mollin and Booth (7) described normal disappearance in a similar group. Patients with steatorrhea are probably a too complex and heterogenous group to be used as examples of vitamin B_{12} deficiency and as Mollin and Booth (2) indicated, the low plasma B_{12} in folic acid deficiencies may not represent tissue deficiency.

The purpose of the present study was to determine whether this abnormality of plasma B_{12} disappearance is related to the deficiency of vitamin B_{12}, or to some permanent defect in patients with pernicious anemia. The two groups selected to represent vitamin B_{12} deficiency were patients with untreated Addisonian pernicious anemia and patients with untreated anemia due to *diphyllobothrium latum* infestation. The former group had both a permanent defect of B_{12} absorption plus a B_{12} deficiency. The latter had only the deficiency. A group with treated pernicious anemia who would have any permanent defect associated with the disease but not the B_{12} deficiency was included. A group of patients infested with fish tapeworm but free of B_{12} deficiency and a group of patients with cardiovascular or skin disease served as controls.

Materials and methods

Subjects

Untreated pernicious anemia 5, 4 female and 1 male, mean aged 69. Treated pernicious anemia 5 all female, mean age 59. Duration of disease of treated group, 8—51 months and

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Table I Plasma B_{12} disappearance Mean values for each group

Group	No. of cases	RBC m/mm³	Plasma B_{12} $\mu\mu g/ml$	Percentage of ⁵⁷Co remaining² at 5	30	60′	90′	6 hr	24 hr.
Control	4	—¹	284	43	23	17	15	11	7.6
Pernicious anemia untreated	5	2.0	82	44	28	25	23	19	14
Pernicious anemia in remission	5	3.8	287	36	19	16	14	9.0	7.4
Tapeworm anemia	5	1.5	80	34	23	20	19	16	12
Tapeworm carrier	5	4.4	502	37	22	17	15	10	8.2
Pernicious anemia in relapse after blocking dose³	2	2.6	746	32	15	11	8.4	4.8	2.5

¹ Mean Hct = 49
² 100% = Calculated time zero concentration.
³ 1 000 μg cold" B_{12} i. m. 3 days before test.

interval between last B_{12} injection and study 18—33 days. Tapeworm anemia. 5, 4 male and 1 female, mean age 52 The diagnosis of all patients in tapeworm anemia group was distinguished from Addisonian pernicious anemia by the presence of *diphyllobothrium latum* ova in the feces and by a clinical response produced by removal of the worm. Tapeworm carriers 5 4 female and 1 male, mean age 58 Controls 4 all male, mean age 65 Two additional patients with untreated pernicious anemia received 1 000 μg of nonradioactive vitamin B_{12} 3 days before the study

Techniques

Cobalt 57 vitamin B_{12}[1] was used in all cases. The usual dose ranged from 0.25—0.76 μg Two subjects, one each from the treated and untreated pernicious anemia groups, received 0.09 and 0.07 μg It was not considered necessary to hold the dose within stricter limits since in our laboratory we have found the same disappearance rate in control subjects over a dose range of 0.04—0.6 μg The radioactivity of the dose ranged from 1.03—2.36 μc of ⁵⁷Co Each lot of Co B_{12} was assayed for radioactivity and also for vitamin B_{12} content b the *Euglena gracilis* z strain, technique. Samples were counted in a well type scintillation detector with a single channel pulse height analyzer This system gave 1.3 × 10^4 c/m/μc of ⁵⁷Co with a background of 28—31 c/m. The counting accuracy of samples was 1—3 The dose was given intravenously in 3.5—5.0 ml of saline, and samples were taken from the opposite arm at 5 25 55 90 6 hr and 24 hr Plasma volume was calculated from the data obtained by Gibson (4) The values of 47.5 ml/kg of body weight for females and 58.9 ml/kg for males were used in untreated pernicious anemia and tapeworm anemia. 43.1 ml/kg for males and 41.5 ml/kg for females were used in the calculations in all non-anemic subjects. The plasma concentration at time zero was taken as

$$\frac{\text{dose given } (\mu c)}{\text{calculated plasma volume (ml)}}$$

[1] Supplied through the kindness of Doctor W P Freeborn, Merck, Sharp and Dohme Research Laboratories, New York N Y

Results

The percentage of the zero time plasma concentration remaining at various times is given in table I The control group showed the expected sharp drop in plasma radioactivity over the first 30 minutes followed by a slower rate of fall Less than 20 % of the activity remained at 1 hour and less than 10 % at 24 hr The initial rate of fall was similar in the untreated pernicious anemia group but

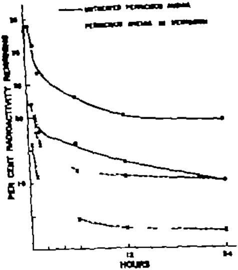


Fig. 1 Maximum and minimum plasma disappearance curves of untreated pernicious anemia group and treated pernicious anemia group.

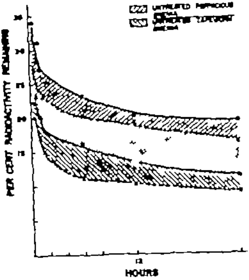


Fig. 2. Maximum and minimum plasma disappearance curves of untreated pernicious anemia group and tapeworm anemia group.

from 30 minutes on a greater amount of activity was retained. The 24-hour re tention was almost twice that of the control group. There was no abnormality in the group with pernicious anemia in remission. The maximum and minimum disappearance curves of both of these groups are shown in fig 1

The results in the group with fish tapeworm anemia were comparable to the pernicious anemia group in respect to severity of illness and plasma vitamin B_{12} concentration. The early portion of the disappearance curve was similar to that of the control group but after 1 hour retention was greater as with the per nicious anemia group. The maximum and minimum disappearance curves of the two B_1 deficiency groups are shown in fig. 2. The group with tapeworm in festation w thout B_1 deficiency or anemia showed no abnormality

The single "blocking" dose of non-radioactive B_{12} given to 2 patients with pernicious anemia in relapse 3 days before the test markedly altered the rate of plasma disappearance of the test dose (table I)

Discussion

The disappearance of the test dose was delayed in patients with untreated per nicious anemia but not in patients with the same disease whose B_{12} deficiency had been corrected either by long term treat ment or by a single large dose shortly before the test. A similar abnormality was found in patients with tapeworm anemia who like those with pernicious anemia had a vitamin B_{12} deficiency but did not have the same permanent in trinsic abnormality the defect in the mechanism for absorbing B_{12}. Tapeworm carriers who were infested with the same parasite but had escaped the B_1 deficiency showed a normal plasma disappearance. These findings suggest that the delayed

disappearance was related to the tissue and/or plasma B_{12} deficiency and not to either some permanent defect associated with pernicious anemia or to the presence of *diphyllobothrium latum*. It seems likely that the fundamental abnormality was either an increased binding of B_{12} by some plasma protein an abnormally firm binding a defective tissue extraction, or a shift of B_{12} back to plasma from tissue. There is no evidence to indicate which of these processes was involved.

The results of this study pertaining to treated pernicious anemia do not agree with those of Brody et al (3) and there is no obvious explanation for the lack of agreement. It should be noted that the method for calculating the 100 % plasma concentration of the test dose used in the present study and in the other studies referred to is not precise for two reasons. There is some loss before mixing is complete (5) and the plasma volume used in the calculations was estimated and not measured A measured plasma volume was used in a previous study (6) and thus did not appear to increase the validity of interpretation over that which could be obtained by calculating plasma volume. The method of treating the data used in the present study was applied consistently to all of the groups to be compared and was considered adequate for the purposes of the study

Summary

A delayed disappearance of an intravenous dose of ^{57}Co B_{12} was found in untreated pernicious anemia and to a lesser degree in the anemia produced by *diphyllobothrium latum*. It was not found in treated pernicious anemia or in tapeworm carriers. The abnormality appears to be due to vitamin B_{12} deficiency and not to any permanent defect associated with the disease pernicious anemia.

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Complications of Carotid Angiography in Acute Cerebrovascular Conditions

By

M Eiken and J Gormsen

Since Moniz (1927) introduced carotid angiography there have been consider able changes in both investigative techniques and indications.

Whilst originally contrast medium was injected into the operatively exposed carotid artery in 1936 the method of percutaneous injection was introduced, which is now used almost exclusively A few investigators recommend general anaesthesia, but the investigation is commonly carried out under local anaesthesia.

During the early years sodium iodide was commonly used as contrast medium, but proved to be too toxic. It was replaced by thorium dioxide (Thorotrast) which, however had to be abandoned, primarily because of the potential danger of radiation injury due to incomplete elimination from the body Since 1939 number of complex iodine derivates have been used, first the di-iodo-pyridone derivates such as Diodrast, Perabrodil, Umbradil, etc., and later tri-iodo-benzoic acid derivatives eg Miokon, or benzoates eg Urografin Hypaque etc. These last named tri-iodo-compounds have generally been found, both by clinical observation and by experimental investigation, to be less toxic than the di-iodo-compounds (Grote & Bettag 1955 Neuenschwander & Renfer 1956 Margolis et al. 1958 Tonnis & Schiefer 1959)

The incidence of complications has been extremely varied. A few authors including Ver Brugghen (1954) and Sedzimir (1955) have not observed a single complication, whilst others report a complication rate of 10—19 per cent (Dunsmore et al 1951 Perese et al. 1954 Bergerhof & Frowein 1955 Coddon & Krieger 1958) or even higher The mortality rate has varied between 0 and 3 per cent. These variations must be attributed partly to differences in the types

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Table I Age distribution and incidence of complications

Age	No. of invest.	Complications		
		Cerebral	Other	Total
<51	51	1	3	4
51—60	67	1	0	1
61—70	146	2	7	9
>70	96	7	6	13
Total	360	11	16	27

of cases selected for investigation, in indications for investigation, and in the time at which it is carried out and partly to the technique used and the skill of the investigator but first and foremost to differences in the interpretation of the term complication A direct comparison of the various reports is therefore not possible.

Although the great majority of previous reports have been based mainly on patients with neurosurgical disorders many have contained warnings against the use of cerebral angiography in such conditions as cerebral thrombosis (Rowbotham et al 1953 Sedzimir 1955) old age and atherosclerosis (Dunsmore et al 1951 Schölzel 1958 Tönnis & Schiefer 1959 and others) The existence of a connection between cardiovascular status, old age and complications to angiography has been to some extent denied by other authors (Kaeser & Thomas 1954 Rishede 1957 Coddon & Krieger 1958 Rupprecht & Scherzer 1958, 1959) and our own experience is also in the main not in agreement with the first mentioned reports. In this paper the complications of carotid angiography which were observed in a series of patients, the majority of whom had been admitted after an apoplectic insult, will be reported.

Material

The investigation includes all the patients from two Medical Units of the Copenhagen County Hospital, Gentofte, on whom carotid angiography was carried out during a three-year period (1958—1961) Altogether 360 carotid angiographies were carried out — including 9 bilateral angiographies at the same session — on 293 patients, 171 men and 122 women. The ages of the patients ranged from 17 to 83 years, with an average of 62 years (table I)

The investigations were carried out by the percutaneous method under local anaesthesia. Otherwise the only comment on technique is that a puncture needle with a blunt stylet, as recommended by Buchtala & Jensen (1955), was used for the punctures. After arterial puncture the stylet is introduced with the rounded end protruding 1 mm beyond the point of the needle. The needle can now be pushed further into the artery with a reduced risk of intimal damage. Where possible the common carotid artery was punctured, and in only a few cases was the contrast medium injected into the internal carotid. During the period reported only two contrast media were used, viz. the chemically closely related Hypaque (Wintrop) 45 and Urografin (Schering) 60 % in 73 and 287 cases, respectively Without having any statistical evidence it is our impression, as also stated by Broadbridge & Leslie (1958) that Urografin gave rise to fewer subjective complaints, in particular with regard to the burning sensation well known from every type of rapid intravascular injection of contrast media.

Nearly all the investigations included frontal and sagittal projections, together with an oblique transorbital view 5—8 ml contrast being injected for each view Frequently a special film was taken of the cervical portion of the carotid artery the total amount of contrast injected being 30—40 ml.

Complications

In connection with the 360 investigations up to 27 complications were recorded (table II) It was characteristic that most of the cerebral complications were first observed after the patient had returned to the ward. On the other hand all the complications, with

the exception of the laryngeal paralysis, were apparent within 24 hours of angiography. Further observation did not reveal any deterioration in the clinical condition which could be attributed to the angiography.

Cerebral symptoms

Cerebral complications such as hemiparesis, sensory disturbances, convulsions and clouding of consciousness have been observed by various authors in connection with about 10 per cent of investigations (Dunsmore et al. 1951; Rowbotham et al. 1953; Perese et al. 1954; Coddon & Krieger 1958. Broadbridge & Leslie 1958. Bull et al. 1960 etc.)

Pathogenetically from the animal experiments of Broman et al. 1948—56, they would appear to be due to direct action of the contrast medium on the tissues, in the first place on the endothelium of the vessels, giving rise to an injury of the blood-brain barrier with increased vascular permeability in severe cases with the development of oedema, stasis, and punctate haemorrhages. These observations have been confirmed and strengthened by Smith et al. 1951. Utterback & Haymaker 1952. Bloor et al. 1951 a, b. Bassett et al. 1953 and others. Electroencephalographic changes, interpreted as indicating direct toxic action on the neurones, are described by Bloor et al. 1951 and Foltz et al. 1952, but Maass & Lennartz (195.) and Greitz & Weiss (1959) were not able to demonstrate any such changes.

T produce demonstrable chemotoxic change in experimental animals it is, however necessary to allow contrast medium of high concentration to affect the vessel wall for rather a long period of time. The concentration of the contrast used, the size of the single dose, the speed of injection and — on account of summation effect — the total number of injections and the time intervals between them, have therefore all been put forward as factors which must be assumed to be of significance in the production of chemotoxic effect. Further there is obviously considerable ariation between the toxicities of the different contrast media.

However as carotid angiography is usually carried out in man, these factors alone are unlikely to be capable of producing cerebral complications of clinical importance. If, however transient reduction in the speed of the blood flow should occur during the investigation, prolonged contact between the contrast medium and the vessel wall might possibly increase the risk of injury. Such reduction in the rate of blood flow could have several causes, viz. spasm of the carotid, provoked by palpation of the neck or per

Table II. Complications of carotid angiography (360 angiographies in 293 patients)

	Complications	
	Transient	Permanent
Cerebral complications		
Aggravation of neurological signs in patients whose symptoms were progressive before angiography — (4 patients)		
Hemiparesis	1	1
Disturbance of consciousness	—	1
Dysphagia	—	1
Aggravation of neurological signs in patients whose neurological condition before angiography was stationary — (6 patients)		
Hemiparesis	5	—
Aphasia	1	—
Epileptic seizures	1	—
Local complications		
Paralysis of right recurrent laryngeal nerve	—	(1)
Haematoma of the neck — (9 patients)		
with dysphonia	1	—
with respiratory arrest	1	—
without other objective findings	7	—
Sensitivity and general reactions		
Nausea, vomiting	2	—
Deterioration of cardiac status (heart failure)	2	—
Collapse during angiography	2	—
Complications total (27 patients)	23	4

ticularly by the direct irritation of the needle chemical vasospasm produced by the contrast medium transient obliteration of the lumen of the artery due to paravasal or intramural injection of contrast, or to bleeding thrombosis at the site of puncture as well as the presence of an arterial obstruction central to the site of puncture raised intracranial pressure, or probably in some cases a circulatory collapse of cardiac origin.

In 11 cases (table II) we observed a change in neurological status during or following angiography but in 10 of these this was only an aggravation of symptoms which were already present, i e hemiparesis, disturbance of consciousness, aphasia, or dysphagia. In 7 of the cases the deterioration was transient, and in the remaining 3 cases, in whom it was permanent, the neurological symptoms had been progressive before angiography so that the relation to the procedure is rather uncertain. As can be seen from table II these cases comprised one of hemiparesis, one of disturbance of consciousness, and one of dysphagia. In one patient epileptic seizures occurred during and intermittently for 24 hours following angiography but the condition of the patient was not otherwise affected.

In several of the patients, particularly those in the older age groups, a slight clouding of consciousness was observed during the first 24 hours after investigation. It is uncertain how far this is to be interpreted as a real complication of angiography as it seemed to resemble more closely an effect of the premedication Severe mental confusion, as those reported by Olsson 1949 1950, Lindgren 1950 Kaplan & Walker 1954 and Bergerhof & Frowein 1955 in some cases even with a fatal course, was never observed amongst our patients.

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Bleeding from the puncture site must be presumed to occur in almost all carotid angiographies (Rimpau & Seils 1957) but in the majority of cases the haematomas are small and harmless. Occasionally they can, however, be so large that they cause compression of the trachea or larynx, and in some cases it has been found necessary to evacuate them, or to carry out tracheotomy or intubation (Fink & Stein 1952 Perese et al. 1954 Albrecht 1955 Dimant et al. 1956 Zoltán 1957) One case in which death occurred was reported by Dunsmore et al in 1951 As suggested by Berdal & Emblem (1951) and later by Rimpau & Seils (1957) these larger haemorrhages must be taken as being in many cases due to simultaneous puncture of the great neck veins, in particular the internal jugular vein

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and transient subjective discomfort. In one case the haematoma was accompanied by a transient dysphonia, whilst in second case, where the contrast medium had by mistake been injected into the vertebral artery transient respiratory arrest, with the development of cyanosis, occurred, requiring intubation. In both cases it is probable that the complication was due to compression of the trachea. In two cases a haematoma occurred in association with other complications, viz. deterioration in the cardiac condition of a patient with hypertension, and slight deterioration of an already present hemiparesis, respectively In neither case did the haematoma appear to have any relation to the other complication.

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It is obviously reasonable to expect a higher risk of such complications in older patients with more or less pronounced degenerative changes in the vessels. I spite of several control investigations we have never however observed any changes demonstrable by angiography which could be attributed to previous carotid puncture. It should be mentioned that Rimpau & Seils, during their pathologico-anatomical investigations, were likewise unable to demonstrate thrombus formation at the site of puncture in any case.

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Symptoms of lesions or irritation of the nerves in the neck in association with percutaneous carotid angiography have otherwise only been reported in relatively few cases, and have apparently always been transient. Paralysis of the recurrent laryngeal nerve or vagus has been reported by Kloss & Radlinger 1950, Ecker 1951 Abbott et al. 1952, whilst lesions of the brachial plexus have been described by Kaplan & Walker 1954 and Bergerhof & Frowein 1955. Horner' syndrome after percutaneous injection is described by Engeset 1944, Wilkinson et al. 1949, Abbott et al. 1952, Bergerhof & Frowein 1955, and both Wilkinson et al. and Abbott et al. have also observed paralysis of the cervical sympathetic chain. Finally several authors, including Kaplan & Walker (1954) and Albrecht (1955), have described transient pupillary dilatation of uncertain origin.

With the local complications should also be included one case of osteomyelitis of the 5th cervical vertebra (Fink & Stein 1952) and possibly according to Ecker (1951) laryngospasm.

Sensitivity and general reactions

Sensitivity reactions such as nausea, vomiting, coughing, sneezing, urticaria, asthmatic attacks, laryngeal oedema, and collapse have been described, but play only very small part amongst the complications. The number of such reactions seems to have been further reduced by the introduction of the various tri-iodo-benzoic acid derivates as contrast media. Decker (1960), who used Urografin, judged the incidence of urticaria, which he considered to be the most common sensitivity reaction, to be approximately 1 per 1,000 investigations.

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In table III are reproduced both the total incidence of complications and the number of cerebral complications in relation to the cerebral status of the patient.

In addition to the 13 patients, in whom it was only possible to demonstrate cerebral atherosclerosis, angiography revealed atherosclerotic changes in the majority of the group with infarcts similarly in 70 of these patients it was possible to demonstrate a total or a severe partial occlusion of the internal carotid or of one of its branches. In 25 of the 27 cases classified as tumours the diagnosis was confirmed either at operation or autopsy As mentioned by Therkelsen (1961) such intracranial neoplasms are not rarely disclosed by cerebral angiography on patients with acute apoplectic symptoms. The diagnosis of haematoma was similarly confirmed in 11 cases, whilst in 5 others the presence of blood in the CSF made the diagnosis extremely probable

It is apparent from the table that in none of the diagnostic groups was there any excessive number of complications.

The last column of table I shows the incidence of complications in the different age groups. Despite the small number of complications it is striking that no less than 7 of the 11 cerebral complications occurred in patients of over 70 years of age. In contrast there was no accentuation of complications in the 61—70 year age group.

Heart failure must be accepted as playing significant role in many cases of cerebral circulatory insufficiency (Thygesen 1961 Gormsen et al. 1961) A summary of the connection between symptomatic heart failure and cerebral or other complications is to be found in table IV It is noteworthy that cerebral complications are three times as common in patients with heart failure whilst other complications occur with about equal frequency in both groups. In all cases the cerebral complications occurred in patients with coronary sclerosis, with or without occlusion. Neither auricular fibrillation, which was recorded in 30 patients, nor incompensation played any part in the development of complications in the present material. On the other hand 5 of the heart patients who developed complications were also hypertensive.

Patients were regarded as being hypertensive if, in addition to diastolic blood pressure of over 110 mm Hg, they also showed hypertensive retinal changes. 141 of the investigations were carried out in patients with hypertension, and it can be seen from table V that the incidence of complications was slightly raised in this group. The development of haematomas in the neck, which could be expected to occur more frequently in these patients, did not, on the contrary give rise to any problems.

As in the present investigation only 11 patients with cerebral complications were recorded in all, it is obvious that the same complications must be repeated in the groups of patients with old age (table I) heart failure (table IV) and hypertension (table V) It appears that amongst 10 of these 11 patients with cerebral complications there were 7 with heart failure and 6 with hypertension, whilst 7 were over 70 years of age. The eleventh cerebral complication occurred in 44-year old man without cardiovascular symptoms. As heart disease and raised blood pressure are

Table IV Complications and cardiac condition

Cardiac condition	No. of invest.	Complications		
		Cerebral	Other	Total
+ heart failure	126	7 (5.5%)	6 (4.8%)	13 (10.3%)
— heart failure	234	4 (1.7%)	10 (4.3%)	14 (6.0%)

Table V Complications and hypertension

Blood pressure	No. of invest.	Complications			
		Cerebral	Haematoma of the neck	Other	Total
+ hypertension	141	6	2 (+ 1)	5	13
— hypertension	219	5	6	3	14

Haematoma developed secondarily during treatment of an attack of dyspnoea, see text.

Table III Complications and cerebral disorder

Cerebral disorder	No. of invest.	Complications	
		Cerebral	Total
Cerebral infarct	274	10	19
Intracranial haematoma	26	0	2
Cerebral tumour	27	1	2
Cerebral atherosclerosis (without clinical infarct)	13	0	0
Aneurysm	5	0	2
Epileptic symptoms	6	0	1
Miscellaneous	9	0	1

pulse and of respiration occurred during haemostatic compression of the carotid artery possibly due to the carotid sinus reflex. The other occurred in a very ill patient, on whom the investigation was, exceptionally, carried out under general anaesthesia. Immediately after angiography there was a considerable fall in blood pressure. In both cases the symptoms remitted rapidly and there were no after-effects.

The post angiographic course in two patients with severe heart failure will be described in more detail. One was a patient with a fresh cardiac infarct, increasing heart failure, and clouding of consciousness. There was no demonstrable reaction to angiography which revealed the presence of a widespread subdural haematoma. Death occurred 30 days later and post mortem examination confirmed the angiographic diagnosis. On account of the progressive deterioration of the clinical condition this case was recorded as a possible complication, although no clinical or pathological relationship between the angiography and the course of the disease was demonstrated. The second patient suffered from severe hypertensive heart failure. Towards the end of angiography dyspnoea occurred. Only after the investigation had been broken off did a visible haematoma appear in the neck. As the patient had had attacks of dyspnoea before the investigation, it is probable that this attack was due to a transient cardiac insufficiency.

The fact that intravascular injection of contrast medium can affect respiration, blood pressure, and cardiac action both in man and in experimental animals, is known from a number of investigations (vide Cotrim 1954, Greitz 1956). Cotrim was able to produce respiratory arrest, arrhythmias, A—V block and sino-atrial delay in dogs by the intravenous injection of very large doses of contrast. He found that the intensity of the reaction depended on the amount of contrast, the speed of injection, and partially on the osmotic pressure of the compound injected. Kågström et al. (1958, 1960) and Lindgren & Törnell (1958) have investigated the effects of the intracarotid injection of relatively small amounts of contrast in dogs and cats, and have demonstrated a vasodilatory effect on the cerebral vessels, but only a sporadic effect on the systemic circulation. These investigations suggest that both slight bradycardia and also hypotension are mainly due to a direct effect of the contrast medium on cerebral vasomotor structures. The experimental animals showed considerable individual variation, and similarly the severity of the reactions to the different contrast media varied greatly. The results were in good agreement with Greitz's (1956) observations on carotid angiography in man.

As a rule the effect on the blood pressure, cardiac action, and respiration appears to be of no practical significance, and as yet no definite relationship between these side effects and clinical complications have been demonstrated. However, Decker (1960) believes that irritation of the vagus in patients with arrhythmias could be thought to compromise the necessary minimum circulation. It should be mentioned that Kågström et al. (1960) on comparing their own results with those reported by Broadbridge (1956) have pointed out a correlation between the strength of the vascular activity and the degree of clinical discomfort, produced by the contrast media.

The relationship of complications to cerebral disease, old age, and cardiovascular condition

Among the conditions which have been stated by several authors to be relative or absolute contraindications to carotid angiography are, as previously mentioned, old age, atherosclerosis, heart failure, and hypertension.

Haematoma of the neck, together with one case of recurrent laryngeal nerve paresis, was the only recorded local, mechanically determined complications. In only two cases did haematomas cause any serious inconvenience when they were accompanied by transient dysphonia and respiratory arrest, respectively. The latter necessitated treatment by intubation.

Sensitivity reactions have not played any practical role. It is usual to call these reactions allergic, despite the fact that the majority of cases are certainly due to idiosyncrasy and that true allergic reactions are seen only very occasionally. Previously extensive testing for sensitivity was carried out, usually by injecting 1—2 ml contrast medium intravenously before the investigation. However such tests have proved to be of questionable value, and are today seldom performed.

Respiratory or circulatory collapse occurred in four cases, but in none did they appear to be general reactions produced by angiography.

The main purpose of the present investigation has been to demonstrate the dependence of the incidence of complications on old age and cardiovascular condition. An account of the complications gives a maximum frequency of 7.5 per cent, of which 3 per cent were cerebral. In only 5.8 per cent was a probable relationship demonstrable between angiography and the complication. These results are not very different from similar accounts of neurosurgical materials, and, indeed, lie at the lower level of these.

An analysis of the above data has revealed a definite preponderance of complications amongst elderly patients with hypertension and heart failure, without making it possible to determine whether one or more of these factors has been decisive. In consideration of the frequently valuable differential diagnostic data which may be produced by these investigations (Therkelsen 1961, Eiken 1961) and which can be of importance in judging both prognosis and therapy, it must be considered justified and desirable to carry out carotid angiography on patients with apoplectic insults of uncertain origin.

Summary

The complications following 360 carotid angiographies, the majority of which were carried out on patients admitted following apoplectiform insults, are described. In addition, a review is made of reports in the literature of complications following cerebral angiography using the newer contrast media. The total incidence of complications in the present material was 7.5 per cent, of which 3 per cent were cerebral.

The possible aetiological factors in the development of the complications are discussed, and the emphasis as far as cerebral complications are concerned is placed on the toxicity of the contrast medium, together with a retardation of the blood flow through the brain.

The present investigation demonstrates a preponderance of complications amongst atherosclerotic patients over 70 years of age, with heart failure and hypertension, without it being possible to determine to what extent one or more of these factors is essential for the production of the complications. Otherwise, no relationship between complications and age, atherosclerosis, heart failure, or hypertension has been demonstrated, and it is not considered that these conditions constitute a definite contraindi-

Table VI Complications and the prothrombin-proconvertin percentage

p-p value	No. of invest.	Complications		
		Cerebral	Haematoma of the neck	Other
<11	5	0	0	1
11—25	34	1	3	0
26—40	24	2	1	0
>40	30	0	0	0
No anticoagulant therapy	267	8	5	6

both regular accompaniments of old age, it is impossible to determine the significance of the single factors in the development of the complications.

Angiography and anticoagulant therapy

Ninety-three of the 360 cerebral angiographies were carried out on patients who were receiving anticoagulant therapy with phenylindanedione. Table VI shows the relationship between complications and the prothrombin-proconvertin percentage (Owren's method). In most cases this was determined on the morning of the angiography whilst in a few cases it has been arrived at by interpolation. Apart from a very small preponderance of neck haematomas in the patients receiving anticoagulants, there is a uniform distribution of complications throughout the whole material. As the development of large haematomas depends largely on the routine of the investigator the table gives no real impression of the bleeding tendency. It was, however our experience that the period of haemostatic compression had generally to be increased in patients with the lower prothrombin-proconvertin percentages, and in later investigations a value of 15 was established as the minimum at which angiography was carried out. Indeed in adipose patients, in whom compression might be difficult, 25 was the minimum value accepted.

Conclusion

The investigation has comprised complications occurring in all the patients from two medical units on whom carotid angiography was carried out during a three year period. Acute cerebrovascular accidents were the most frequent reason for admission in contrast to the majority of previous reports, which have consisted predominantly of neurosurgical patients. Active heart failure, atherosclerosis, hypertension, and old age have not been considered as contraindications to investigation.

As in previous reports, the complications have been classified in groups according to their nature and presumptive origin. This type of division is to some extent illusionary as both mechanical and toxic reactions in varying combinations must be supposed to contribute to the production of most of the clinically manifest complications.

This is particularly true of cerebral complications, which in this, as in previous materials, have comprised the largest and most serious group with 8 transient and 3 permanent complications. However of these only seven were probably due to the angiography whilst in the remaining four — including all the permanent lesions — the relationship is uncertain, as there might have been a coincidental progression of neurological symptoms already present.

For the development of cerebral complications a retardation of the blood flow in combination with the chemotoxic effect of the contrast medium, is considered to be essential. The horizontal position necessary for investigation must be considered unfortunate in patients with heart disease, and can perhaps contribute towards a transient reduction of the cerebral blood flow

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both regular accompaniments of old age it is impossible to determine the significance of the single factors in the development of the complications.

Angiography and anticoagulant therapy

Ninety-three of the 360 cerebral angiographies were carried out on patients who were receiving anticoagulant therapy with phenylindanedione. Table VI shows the relationship between complications and the prothrombin-proconvertin percentage (Owren's method). In most cases this was determined on the morning of the angiography whilst in a few cases it has been arrived at by interpolation. Apart from a very small preponderance of neck-haematomas in the patients receiving anticoagulants, there is a uniform distribution of complications throughout the whole material. As the development of large haematomas depends largely on the routine of the investigator the table gives no real impression of the bleeding tendency. It was, however, our experience that the period of haemostatic compression had generally to be increased in patients with the lower prothrombin-proconvertin percentages, and in later investigations a value of 15 was established as the minimum at which angiography was carried out. Indeed in adipose patients, in whom compression might be difficult, 25 was the minimum value accepted.

Conclusion

The investigation has comprised complications occurring in all the patients from two medical units on whom carotid angiography was carried out during a three-year period. Acute cerebrovascular accidents were the most frequent reason for admission, in contrast to the majority of previous reports, which have consisted predominantly of neurosurgical patients. Active heart failure, atherosclerosis, hypertension and old age have not been considered as contraindications to investigation.

As in previous reports, the complications have been classified in groups according to their nature and presumptive origin. This type of division is to some extent illusionary as both mechanical and toxic reactions in varying combinations must be supposed to contribute to the production of most of the clinically manifest complications.

This is particularly true of cerebral complications, which in this, as in previous materials, have comprised the largest and most serious group with 8 transient and 3 permanent complications. However of these only seven were probably due to the angiography whilst in the remaining four — including all the permanent lesions — the relationship is uncertain as there might have been a coincidental progression of neurological symptoms already present.

For the development of cerebral complications a retardation of the blood flow in combination with the chemotoxic effect of the contrast medium, is considered to be essential. The horizontal position necessary for investigation must be considered unfortunate in patients with heart disease, and can perhaps contribute towards a transient reduction of the cerebral blood flow.

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Catabolism and Distribution of Albumin in Gastric Cancer

By

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It has been shown that normally albumin degradation probably occurs mainly in the upper part of the gastrointestinal tract in man (Birke et al. 1959) Further quantitative information on this catabolism was presented by Wetterfors et al. in 1960 Experimental studies in animals have since provided additional supporting evidence for these observations (Armstrong et al. 1960, Ullberg et al. 1960, Campbell et al. 1961)

In "exudative gastroenteropathy" an increased permeability of the alimentary tract to high molecular substances, for instance albumin, has been demonstrated (Citrin et al. 1957 Gordon 1959 Schwartz & Jarnum 1959 Holman, Nickel & Sleisenger 1959)

An analogous process occurs in patients with regional enteritis and ulcerative colitis (Steinfeld et al. 1957 Steinfeld et al. 1960 Birke et al. 1961 Wetterfors et al. 1961)

Cancer of the stomach is another disease of the gastrointestinal tract that often gives rise to hypoalbuminaemia. The genesis of the latter is not completely clear but an increased leakage of albumin through the stomach in gastric cancer has been demonstrated earlier as has an increased breakdown (Birke, Liljedahl Plantin & Wetterfors 1959)

The present investigation — one of a series concerned with protein metabolism in man under normal and pathological conditions — will try to throw further light on the problem of hypoalbuminaemia in cancer of the stomach.

Material

Twenty-three patients (11 women and 12 men) 31—78 years old were examined. 20 had gastric cancer of different degrees of severity and one of these (case 20) had cancer of the cardia. No cases of heavy bleeding or acute bleeding occurring during the investigation were included. Only 2 patients had slight anaemia.

Three patients (cases 21 22, 23) who had earlier undergone total gastrectomy because of gastric cancer were also examined at varying times after the operation.

In 6 of the cases the cancer was assessed as operable from the clinical and roentgenological point of view (cases 1—6) Partial or total gastrectomy was performed in 4 while one patient refused operation, and in

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of the original anamnestic weight (mean value) is of about the same order of magnitud (7—9 %). The weight-losses within groups, on the other hand, are very unevenly distributed among the individual patients (table I).

The controls consist of 15 patients (7 women and 8 men) with a mean age of 39 years (19—65 years). They had been admitted to the surgical and medical clinics for investigation of symptoms of no significance in relation to this study (12 cases) or had undergone minor operations, such as hernioplasty or meniscectomy (3 cases). In the latter group the studies were started later than 7 days after the operations.

Methods

^{131}I-labelled albumin

In the first part of this study ^{131}I-labelled albumin from the Radiochemical Centre Amersham, England, was used. This albumin contains one atom of iodine per albumin molecule. The radioactivity per mg albumin is 10—15 μC. This preparation was used in the first 7 cancer-cases, in one of the cases in which total gastrectomy had been performed, and in the 5 earliest control cases.

In the other 13 cancer-patients, in the other 2 totally gastrectomized patients, and in ten controls ^{131}I-albumin from Abbott (RISA-AI) was used. This preparation contains approximately one atom of iodine per six albumin molecules, and the radioactivity per mg albumin at 5 μC.

Both preparations of radioactive albumin were controlled by dialysis for their content of free ^{131}I-iodide. This was below 1 % of the total activity. Controls by paper-electrophoresis showed that the ^{131}I-albumin preparations behaved in vitro as non-radioactive albumin. To avoid as far as possible the self-irradiation effect (Cohen 1959, Wetterfors et al. 1960) the preparations were used within 3 weeks after delivery.

The administered doses varied between 20 and 60 μC. The radioactivity was measured in 2-ml specimens of whole blood, plasma, ascites fluid, urine and gastric juice. Faecal specimens were assayed directly in special tins, and the radioactivity was calculated by comparison with a known standard whose activity had just been determined by the same technique.

The specimens were assayed in a well-type scintillation counter Tracerlab P 20 BW connected to a Tracerlab "Superscaler SC-18A. Corrections were made for background activity which was determined each day and also for radioactive decay by reference to a standard. In the assay counts of sufficient number to ensure an accuracy of at least ± 2 % were registered. Towards the end of the study of some patients in whom there was a rather rapid fall of intravascular radioactivity the accuracy was ± 5 in blood samples and ± 10 % in urine samples.

Blocking of the thyroid gland

In each case the thyroid gland was blocked with Lugol's solution (10—15 drops three times daily for 1 to 3 days before and 4 to 5 days after the injection of labelled albumin). Thyroidal uptake was measured for the first 4—5 days. A scintillation counter with clinical collimator (LKB-Produkter Stockholm) was used for this purpose. All measurements were corrected for background activity and referred to a standard.

Earlier it was suspected (Wetterfors et al. 1960) that the activity over the thyroid (measured with a large collimator) of 3—14 % could be ascribed to insufficient blocking, since the difference in activity between the neck and the groin was great. The following investigation showed that this was not the case. With a small collimator lower values for the radioactivity over the neck (2—6 %) were obtained. In two cases of total thyroidectomy activities of the same order of magnitude as in blocked, not thyroidectomized, cases were registered after intravenous injection of ^{131}I-albumin. In two cases of goitre total body scintigram taken after 2 days of Lugol medication and subsequent injection of ^{131}I-albumin showed very small uptake of radioactivity over the thyroid gland. On the following day thyroidectomy was performed, and the activity in the resection specimen was measured under the same conditions as in vivo: 0.1—1 % of the given dose appeared in the extirpated gland. Accordingly the measured activities over the neck are mainly referred to body background.

Table I Certain clinical data for the investigated material. In four cases no data for the weight loss could be obtained

Case	Sex	Age	Body length	Body weight	Weight loss in % of pre mor bid weight
Operable cases					
1	♀	70	154	45	25
2	♀	68	152	68	0
3	♀	66	164	72	4
4	♂	62	165	57	12
5	♀	54	156	47	6
6	♂	72	174	63	—
	Mean	65	161	58.6	7.8
Inoperable cases without ascites					
7	♂	68	176	63	11
8	♀	67	151	41	—
9	♂	67	175	63	0
10	♀	51	162	40	28
11	♂	54	168	58	9
12	♂	31	182	72	0
	Mean	56	169	56	8.0
Inoperable cases with ascites					
13	♂	65	173	65	0
14	♂	55	175	52	22
15	♂	32	175	50	23
16	♀	60	158	65	0
17	♀	56	163	44	4
18	♀	71	160	57	—
	Mean	57	167	56	8.3
Ascites (cancer) but no cancer ventr left					
19	♀	72	163	36	—
Operable cardiastenosis (cancer)					
20	♀	61	167	62	27
Total gastrectomy for cancer ventr					
21	♂	61	170	61	—
22	♂	78	165	48	—
23	♂	55	178	72	—
Controls n = 13	Mean	39	170	66.5	—

one severe arteriosclerosis was a contra-indication to operation. Also the stenotic cancer of the cardia in case 20 was judged as operable.

In the remaining 13 patients (cases 7—19) the cancer was inoperable. In 7 the inoperability was verified by laparotomy long before or after the examination with ^{131}I labelled albumin. In 3 of the patients with inoperable cancer early explorative laparotomy was indicated and performed during the investigation (cases 16, 17 and 18). In the rest of the cases the inoperability was clinically and roentgenologically clear. Metastasis to lymph-nodes or spread of the tumour to surrounding tissue was present in all these patients. Liver metastases was verified in one patient (case 14). Ascites of varying degree occurred in seven patients (cases 13—19). One patient (case 14) had virtually complete stenosis of the gastric antrum, due to the cancer and one patient (case 10) with a recurrent scirrhous carcinoma in the cardia region had almost complete stenosis of the cardia.

One patient (case 19) who had 8 years earlier undergone gastric resection had roentgenological changes which strongly suggested cancer. Ascites was also present. Laparotomy revealed that there was no cancer in the gastric remnant but the patient had metastases in the abdominal cavity from the removed gastric cancer. This patient was examined preoperatively.

Thirteen patients received neither blood nor plasma, nor albumin while they were being investigated.

Ten patients (see fig. 1 a—c) received transfusion of blood, plasma or albumin on medical grounds during the period of investigation. Fig. 1 also shows the fluctuations in the relative serum-albumin concentration.

The eventual significance of the influence of this replacement therapy and of the surgical interventions in the investigations will be discussed later (see Discussion).

In case 16 laparocentesis was performed twice.

In two patients (cases 4 and 20) nitrogen-balance studies were made for 8 and 10 days, respectively, in connection with ^{131}I-albumin studies.

The mean weight-loss in each group of investigated patients expressed as a percentage

a steady state, i. e. that the elimination constants for intravascular activity and retained dose are equally large.

Dialysis of plasma and ascitic fluid

In 6 of the cancer-patients (cases 4—6, 11 12 and 18) and in 8 of the controls dialysis of plasma was made for 24 hours and the amount of dialysable radioactivity that could be precipitated with silver nitrate ($AgNO_3$) was determined.

In 3 patients (cases 18, 19 and one case which otherwise was excluded because of bleeding) dialysis of ascitic fluid was made for 24 hours to determine the amount of dialysable radioactivity (precipitable with $AgNO_3$).

Gastric juice

Gastric juice was collected through an ordinary duodenal tube. In the first 5 cases this was done a few hours after the injection of ^{131}I albumin, in the other ten on the first to fourth day after injection. In the latter cases collection were made as quantitatively as possibly by rinsing with physiological saline for 1 hour in the achylic patients (5 cases) and with phosphate buffer (pH 7.2 ionic strength 0.2) (Gullberg & Olhagen 1959) in the normochylic patients (5 cases). In some cases the investigation was carried out on the fourth day so that the quantitative calculations would be made as close to supposed equilibrium as possible. In all cases as [illegible] saline or buffer was used, the stomach was first washed out with saline. All contamination with saliva was avoided. Bile-coloured portions of gastric juice were discarded and, instead, the time of aspiration was increased.

By centrifuging the presence of any red cells was revealed. Correction for the presence of blood was thus made in four cases by determining the red-cell count. On the basis thereof and of the number of red cells in blood and haematocrit the corresponding amount of poured-out plasma was calculated. Corresponding correction of the radioactivity was then made. Cases with macroscopically blood-stained gastric juice are not included in the account.

The gastric juice was filtered through glass-wool and centrifuged. Carrier albumin and potassium iodide were added, and the mixture was precipitated with an equal volume of 20 % trichloracetic acid (TCA) and washed with 10 % TCA. From the supernatant the peptides were precipitated with 5 % phosphotungstic acid (PWA) and the free iodide was precipitated with 10 % $AgNO_3$. The radioactivity in the different precipitates was then measured.

Paper-electrophoresis on the ultrafiltrate of gastric juice was done according to Gullberg & Olhagen (1959) modification of Glass method. In most cases electrophoresis strips were cut and the activity in the different protein fractions was measured.

Radioactivity in the stomach wall

In 7 patients (cases 3, 4, 5, 14 and 18 and in two that were not included in the material in other respects because of haemorrhage) the activity per gram of tumour tissue and healthy gastric tissue was measured. In two cases comparison had to be made with the intestine, since no definitely healthy stomach wall could be found. These measurements were made after rinsing with physiological salt solution and drying by moderat pressure between filter papers.

Nitrogen-balance studies

Twenty-four-hour weighed specimens of urine and faeces and specimens of the food after homogenisation were assayed for nitrogen by the Kjeldahl procedure. The factor 6.7 was used for transforming of nitrogen into g of protein.

Statistical methods

The statistical treatment was done by generally accepted methods with calculation of mean-values and standard deviations. Student t-test for small samples (see Snedecor 1956) was used to determine the level of significance.

The following significance levels were used

(x) Probably significant $0.05 > p > 0.01$
(xx) Significant $0.01 > p > 0.001$
(xxx) Highly significant $0.001 > p$.

Results

The results of some of the investigations will be reported for the material of gastric-cancer patients (cases 1—18)

Blood plasma and red-cell volumes

The plasma volume (PV) was calculated by the current isotope-dilution technique.

After the injection of ^{131}I albumin into one arm specimens of whole blood and plasma were taken from the other arm 6 and 9 minutes after the injection.

The highest of the 6 or 9 minute values was used in calculating the plasma volume after correction for extravascular distribution (Veall & Vetter 1958) by the factor 1 010—1 015 (6 and 9 minutes, respectively)

The blood volume (TBV) was then calculated from the formula

$$TBV = \frac{PV \cdot 100}{100 - \text{body haematocrit}}$$

The quotient $\frac{\text{activity/ml whole-blood}}{\text{activity/ml plasma}} \cdot 100$ expresses as a percentage the proportion of plasma 100 minus this value is the venous haematocrit.

Body haematocrit was obtained by correction of venous haematocrit by the factor 0.91 (Chaplin Mollison & Vetter 1953)

The haematocrit values thus obtained agreed well with those simultaneously determined by Wintrobe's method corrected for trapped plasma (0.95) and body haematocrit (0.91) The red-cell volume (RCV) is TBV—PV

In comparing the TBV PV and RCV between the different patient groups and the control group not only the total values were used, but also values referred to body-length and actual body-weight (per metre per kg)

Serum proteins

Total protein was determined by the Kjeldahl procedure, and the different protein fractions by paper-electrophoresis in veronal buffer of pH 8 6, ionic strength 0 1 (see Olhagen 1956)

Normal values for total protein are 6 5—8.0 g per 100 ml and for albumin 4 0—5 5 g per 100 ml (Swedin 1961)

Intravascular albumin

The amount of intravascular albumin was calculated from the albumin concentration and the plasma volume.

In comparing the intravascular albumin between the different groups the same parameters as for the blood-volumes were used.

Distribution and breakdown of albumin

Steady state means in this investigation a constant equilibrium between intra- and extravascular compartments. The expression is thus not used in the metabolic sense of an exact balance between catabolism and synthesis.

Both Campbell's et al. (1956) and Matthews (1957) methods for analysis of distribution presuppose a steady state.

As our patients were not in a steady state (see Results and Discussion) we instead calculated the distribution as follows From elimination curves for both intravascular activity and retained dose the elimination constants for the respective curves (k_{iv} and k_{rt}) were determined from the formula for an exponential function

$$A_t = A_o \cdot e^{-k \cdot t}$$

where A_o is the extrapolated activity at the time 0, A_t the activity at the time t, e the base in the natural logarithm system, and k the desired elimination constant. This formula is valid for the exponential part of the respective elimination curves. In those cases in which it was difficult to construct graphically on the basis of the obtained values the elimination curve that corresponded best to the course, the method of least squares was used for calculating the k values (Odeblad et al. 1959)

To obtain a numerical symbol for the prevailing non-steady state the quotient $\frac{k_{iv}}{K_{rt}}$ was constructed. It gives a relative measure of the divergence in the course of the elimination curves for intravascular and total radioactivity and, thus, also for the pathological distribution. Also the difference (K_{iv}—K_{rt}) was calculated.

The degradation of albumin was calculated by Campbell's et al. (1956) method, where the total radioactivity in a 24-hour urine sample is compared with that in the plasma for each day The calculations are made over 7—8 24-hour periods. This method is valid in calculating the degradation but not, as mentioned earlier the distribution.

Matthews (1957) and Sterling (1951) methods of calculation could not be used, since they are based wholly on the elimination curve for the plasma activity and presuppose

Initial relative hypoalbuminaemia (< 4 g per 100 ml) was present in 16 patients, in 10 of whom it was marked (< 3.6 g per 100 ml)

The hypoalbuminaemia was least marked in the operable cases (3 80 ± 0.34 g per 100 ml) while in the two inoperable groups the mean values were 3.55 ± 0.39 and 3 40 ± 0 48 g per 100 ml, respectively

Fig 1 shows the variations in total protein and albumin concentration in the different groups during the course of investigation, and the amount of albumin administered in those cases in which replacement therapy was indicated on medical grounds.

The variations in the albumin concentration in the operable cases were slight, but in case 5 a decreasing albumin concentration was noted. The decrease was proportionally greater than the one for total proteins. In case 4 on the other hand, there was a rise in total proteins, which was not paralleled by an equivalent rise of the albumin concentration.

In the inoperable cases without ascites the already low relative albumin concentration showed small variations, with a tendency to fall in three.

In the inoperable group with ascites small variations occurred but in those two cases who had received large amounts of albumin there was a slight rise in the albumin concentration.

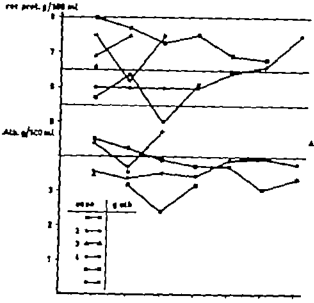
Of the two totally gastrectomized patients (cases 21 and 22) who were investigated 1 year and 10 years, respectively after the operation, one had a low albumin concentration which, however became normal (4 1 g per 100 ml) with three weeks of high-caloric and high-protein feeding The other patient had a normal concentration. The third patient (case 23) who was examined 4 weeks after operation, had still a relative hypoalbuminaemia.

Blood-(TBV) plasma-(PV) and red-cell volumes (RCV)

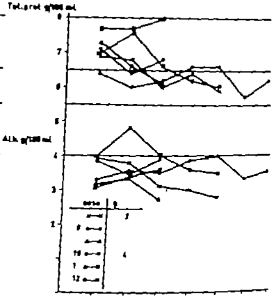
The mean TBV in the whole series of cancer patients (cases 1—18) was 4 46 ± 0.79 l, while the means for PV and RCV were 2.93 ± 0.55 l, and 1.53 ± 0.38 l, respectively These values did not deviate significantly from those of the control cases. Nor were there any significant deviations from the control group when these values were referred to body-length and body-weight

The operable group had a mean TBV that was lower (x) than that in the control group When the TBV was referred to body-length (TBV/m) however there was no significant difference (2.44 ± 0.41 l and 2.79 ± 0 37 l per m, respectively) nor when it was correlated with body-weight. The PV calculated totally or in relation to body length and body weight did not deviate significantly from that of the control group. The RCV on the other hand, was lower (x) both totally (1.20 ± 0.28 l and 1.65 ± 0.39 l, respectively) and per metre (0 75 ± 0.18 l and 0.96 ± 0.19 l, respectively) while per kg it was not significantly lower (table II) This fall of the RCV/m indicates that it is not a seeming but probably a true decrease. It is, however not so marked as to give together with the not significant decrease of PV/m, a significant decrease of TBV/m.

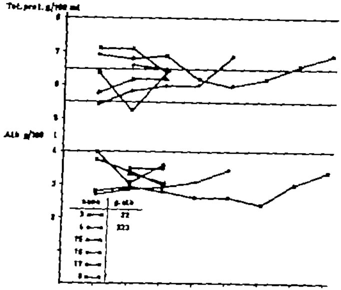
In the group of patients with *inoperable cancer without ascites* TBV PV and RCV did not differ from those in the control group, either in total amounts or in relation to body-length. Referred to



a



b



c

Fig 1 a, b and c. Variations in total protein and albumin during periods of investigation. The intervals between determinations are 3—5 days. The horizontal lines denote Mean ± 2 SD for normals. a operable cases. b inoperable cases without ascites. c inoperable cases with ascites. In each fig are given symbols for the cases and amounts of albumin infused during periods of investigation.
In cases 4 and 5 the two last determinations are made postoperatively

both as a whole and divided into 3 groups operable, inoperable without ascites, and inoperable with ascites. The object of this procedure is to try to find out in what way the extension of the cancer has influenced the measured parameters. It means, on the other hand, that the groups are relatively small, which must be taken into consideration in judging the results.

For the rest of the patients (cases 19 and 20) who could not be referred to any of the above-named groups, the results will be reported separately

Serum-proteins

Among the 20 cancer patients investigated with [131]I-albumin only 6 had initially a slight hypoproteinaemia (< 6.5 g per 100 ml) the lowest values being 6 0 and 5 8 g per 100 ml

Initial relative hypoalbuminaemia (< 4 g per 100 ml) was present in 16 patients, in 10 of whom it was marked (< 3.6 g per 100 ml)

The hypoalbuminaemia was least marked in the operable cases (3 80 ± 0.54 g per 100 ml) while in the two inoperable groups the mean values were 3.55 ± 0.39 and 3 40 ± 0 48 g per 100 ml, respectively

Fig. 1 shows the variations in total protein and albumin concentration in the different groups during the course of investigation, and the amount of albumin administered in those cases in which replacement therapy was indicated on medical grounds.

The variations in the albumin concentration in the operable cases were slight, but in case 5 a decreasing albumin concentration was noted. The decrease was proportionally greater than the one for total proteins. In case 4 on the other hand, there was a rise in total proteins, which was not paralleled by an equivalent rise of the albumin concentration.

In the inoperable cases without ascites the already low relative albumin concentration showed small variations, with a tendency to fall in three.

In the inoperable group with ascites small variations occurred but in those two cases who had received large amounts of albumin there was a slight rise in the albumin concentration.

Of the two totally gastrectomized patients (cases 21 and 22) who were investigated 1 year and 10 years, respectively after the operation, one had a low albumin concentration which, however became normal (4 1 g per 100 ml) with three weeks of high-calorie and high-protein feeding The other patient had a normal concentration. The third patient (case 23) who was examined 4 weeks after operation, had still a relative hypoalbuminaemia.

Blood-(TBV) plasma-(PV) and red-cell volumes (RCV)

The mean TBV in the whole series of cancer patients (cases 1—18) was 4 46 ± 0.79 l, while the means for PV and RCV were 2.93 ± 0.55 l, and 1.53 ± 0.38 l, respectively These values did not deviate significantly from those of the control cases. Nor were there any significant deviations from the control group when these values were referred to body length and body-weight.

The operable group had a mean TBV that was lower (x) than that in the control group. When the TBV was referred to body-length (TBV/m) however there was no significant difference (2.44 ± 0.41 l and 2.79 ± 0.37 l per m, respectively) nor when it was correlated with body weight. The PV calculated totally or in relation to body-length and body-weight did not deviate significantly from that of the control group The RCV on the other hand, was lower (x) both totally (1.20 ± 0.28 l and 1.65 ± 0.39 l, respectively) and per metre (0.75 ± 0.18 l and 0.96 ± 0 19 l, respectively) while per kg it was not significantly lower (table II) This fall of the RCV/m indicates that it is not a seeming but probably a true decrease. It is, however not so marked as to give together with the not significant decrease of PV/m, a significant decrease of TBV/m.

In the group of patients with *inoperable cancer without ascites* TBV PV and RCV did not differ from those in the control group either in total amounts or in relation to body-length. Referred to

Table II TBV PV and RCV totally and referred to the parameters body-length and body-weight, the

Cases	TBV	PV	RCV	TBV/m
Operable n = 6	3.92 ± 0.65	2.72 ± 0.58	1.20 ± 0.28	2.44 ± 0.41
Inoperable without ascites n = 6	4.84 ± 0.94	3.19 ± 0.60	1 65 ± 0.37	2.84 ± 0.38
Inoperable with ascites n = 6	4 62 ± 0.51	2.89 ± 0.44	1.73 ± 0.30	2.76 ± 0.27
19	2.51	2.01	0.50	1.54
20	4.90	3.53	1.37	2.94
21	5.20	3.32	1.88	3.06
22	3.92	2.74	1 18	2.38
23	5.50	3.90	1.60	3.09
Controls n = 13	4 74 ± 0.80	3.10 ± 0.45	1.65 ± 0.39	2.79 ± 0.57

body weight however TBV/kg and PV/kg were significantly (xx) higher than in the controls as was also RCV/kg (x) (table II)

In the group of *inoperable cancer with ascites* there was, equally no deviation from the control group as regards total volumes or volumes per m of body length. When referred to body weight a much greater standard deviation was obtained on calculation of the volumes. TBV/kg and RCV/kg were significantly (x) higher while PV/kg showed a moderate but not significant rise, which is clearly lower than in the group without ascites (table II)

The patients examined postoperatively had slightly increased blood volumes in comparison with the operable group (TBV/m) The same was found for PV and RCV On comparison with the controls there were very moderate differences.

Intravascular albumin

In the 18 patients with gastric cancer the mean value for intravascular albumin was 104 6 ± 22 4 g which means a significant (xx) lowering as compared with the controls. On correlation with body-weight there was no significant change while correlation with body length showed a significant (xx) lowering compared to the control group (63 0 ± 12.5 and 75.2 ± 10 3 g per m, respectively)

The absolute amount of intravascular albumin in each group is lower than in the control group. For the operable group the decrease is probably significant (x) as in the inoperable group with ascites, while for the inoperable group without ascites the values were not significantly lower (table III)

When the intravascular albumin is calculated per kg body weight the mean values obtained for the operable cases and for the inoperable cases without and with ascites do not deviate significantly from those of the control cases (table III)

With correction for body-length (table III) there is a significant (xx) decrease for the operable cases and the inoperable cases with ascites (63.2 ± 11 3 g per m, and 59.2 ± 14 1 g per m, respectively) but no significant decrease for the inoperable cases without ascites (66 8 ±

values represent the Mean ± S. D

PV/m	RCV/m	TBV/kg	PV/kg	RCV/kg
1.69 ± 0.38	0.73 ± 0.18	67.2 ± 4.83	46.3 ± 2.67	21.8 ± 5.3
1.91 ± 0.21	0.97 ± 0.16	87.5 ± 10.4	58.0 ± 8.9	29.5 ± 2.0
1.73 ± 0.27	1.03 ± 0.15	85.4 ± 18.5	53.1 ± 11.0	32.3 ± 9.6
1.23	0.33	69.7	55.9	13.9
2.12	0.82	76.9	55.4	21.5
1.95	1.11	85.5	54.6	30.9
1.66	0.67	81.5	57.0	24.6
2.19	0.90	76.8	54.4	22.4
1.82 ± 0.21	0.96 ± 0.19	72.0 ± 8.08	47.2 ± 5.97	24.8 ± 4.54

13.1 g per m) in comparison with the controls (7.2 ± 10.3 g per m).

The totally gastrectomized patients had on the whole normal values for intravascular albumin calculated per kg body weight and per m body-length.

Albumin degradation

Since the normal values for degradation differ slightly between the Amersham and the Abbott [131]I-albumin preparations, this difference must be taken into consideration in comparing the results. For the Amersham preparation (Am) the mean value for normal albumin catabolism is 13.9 ± 3.29 % and for the Abbott preparation (Abb) 9.0 ± 0.99 % (table IV).

In the group of patients with *operable cancer* albumin catabolism could be calculated in five (cases 1, 3, 4, 5 and 6). In 3 of these it was raised, being 21.2 % (Am), 13.8 % and 11.5 % (Abb) and in two it was virtually normal, or 10.2 % and 10.5 (Abb).

An increased or moderately increased catabolism of 27.0 %, 16.0 % and 19.1 % (Am) was found in three patients (cases 7, 8 and 9) in the group of *inoperable cancer without ascites* while normal values were recorded in two (cases 11 and 12) being 9.9 % and 9.2 % (Abb) respectively. The patient (case 10) with almost complete stenosis of the cardia had very low catabolism, viz. 3.6 % (Abb).

In case 20, the other patient with almost complete stenosis of the cardia, the albumin catabolism was also lower than normal, viz. 7.4 %.

A raised rate of catabolism was throughout noted in the patients *with ascites* (cases 13—18). In cases 13 and 16 the rise was slight, however (table IV).

In the patients examined postoperatively the catabolism was normal except in case 25 who was examined only 4 weeks after operation (18.8 %).

Gastric juice

In 15 cases gastric juice was collected. In 9 of these it was entirely free from blood and in 4 the sediment after centrifuging contained only a very small amount of blood. In two cases the gastric juice was macroscopically blood-stained, and the results in these cases (12 and 15) will therefore not be reported.

Table II TBV PV and RCV totally and referred to the parameters body-length and body-weight; the

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Inoperable without ascites n = 6	4.84 ± 0.94	3 19 ± 0.60	1 65 ± 0.37	2.84 ± 0.58
Inoperable with ascites n = 6	4.62 ± 0.51	2.89 ± 0.44	1.73 ± 0.30	2.76 ± 0.27
19	2.51	2.01	0.50	1.54
20	4.90	3.53	1.37	2.94
21	5.20	3.32	1.88	3.06
22	3.92	2.74	1 18	2.38
23	5.50	3.90	1 60	3.09
Controls n = 15	4.74 ± 0.80	3.10 ± 0.45	1.65 ± 0.39	2.79 ± 0.37

body weight however TBV/kg and PV/kg were significantly (xx) higher than in the controls as was also RCV/kg (x) (table II)

In the group of *inoperable cancer with ascites* there was equally no deviation from the control group as regards total volumes or volumes per m of body length. When referred to body weight a much greater standard deviation was obtained on calculation of the volumes. TBV/kg and RCV/kg were significantly (x) higher while PV/kg showed a moderate but not significant rise, which is clearly lower than in the group without ascites (table II)

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Intravascular albumin

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The absolute amount of intravascular albumin in each group is lower than in the control group For the operable group the decrease is probably significant (x) as in the inoperable group with ascites, while for the inoperable group without ascites the values were not significantly lower (table III)

When the intravascular albumin is calculated per kg body weight the mean values obtained for the operable cases and for the inoperable cases without and with ascites do not deviate significantly from those of the control cases (table III)

With correction for body length (table III) there is a significant (xx) decrease for the operable cases and the inoperable cases with ascites (63.2 ± 11 3 g per m, and 59 2 ± 14 1 g per m, respectively) but no significant decrease for the inoperable cases without ascites (66.8 ±

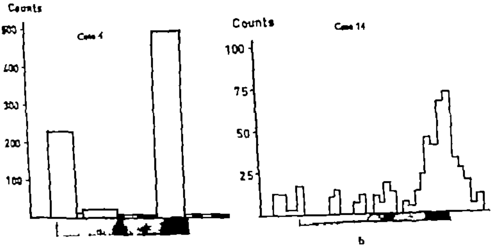


Fig 2. The distribution of radioactivity in electropherogram of gastric juice from two cases of gastric cancer (cases 4 and 14) The maximum of radioactivity is in the albumin area. Some activity has more cathodic localization, probably derived from breakdown products from the albumin.

In the four cases in which the gastric juice was slightly blood-stained the amount of blood varied between 0.07 and 2 mm³ (see Methods) and, thus, it can be estimated that about 0.02 % of the activity in the gastric juice arose from plasma poured out with the bleeding

In all cases electrophoresis showed presence of albumin in the gastric juice, and the radioactivity was concentrated in the albumin-fraction. Fig 2 shows the electropherogram and distribution of radioactivity in two cases.

The precipitation procedure described under Methods was used for measuring the protein-bound radioactivity (TCA + PWA) free radioactivity present as ¹³¹I-iodide ($AgNO_3$) and non-precipitable fraction, most probably ¹³¹I-amino-acids (table V a) Table V a also shows the values for a control group of normochylic and achylic patients (Wetterfors et al. 1960)

The highest values for protein-bound activity as well as for non-precipitable fraction, were obtained in the seven patients with achlorhydria. In the other five patients with normochylia or moderate hypochylia the values accorded with those in the control cases.

In case 19 the gastrectomized patient in whom the radiograms were strongly suspected of showing evidence of cancer in the achlorhydric gastric remnant, only 1 % of protein-bound activity appeared in the gastric juice. Laparotomy showed later that cancer was absent from the gastric remnant.

In the six cases in which both the protein-bound and the non precipitable fraction excreted in the gastric juice (calculated per 60-minute samples) were determined as a percentage of intravascular activity the values showed a relatively great standard deviation (table V b) Transformation of the protein-bound and the non-precipitable activities into quantitative measures (g per 24 hours) gives varying values (approximately 2—6 g) which means that 1/4—1/2 of the amount of albumin catabolised per 24-hours in the respective cases was

Table III Intravascular albumin pools (g) totally and referred to body-length and body-weight Mean ± S D is given in the different groups

Case	Intra-vascular albumin	Intra vascular albumin/m body-length	Intra vascular albumin/kg body-weight
Operable			
1	93	60.4	2.08
2	114	75.0	1.68
3	127	77 4	1 76
4	91	55.1	1.59
5	98	62.8	2.07
6	84	48.3	1.35
Mean ±S.D	101.2 ± 16.2	63.2 ± 11.3	1 76 ± 0.28
Inoperable without ascites			
7	143	81.2	2.27
8	92	60.9	2.27
9	112	64.0	1 78
10	97	59.9	2.42
11	85	50.6	1 47
12	153	84 1	2.12
Mean ±S.D.	113.7 ± 28.2	66.8 ± 13.1	2.06 ± 0.36
Inoperable with ascites			
13	116	67 0	1 78
14	91	52.0	1 75
15	89	50.9	1 78
16	115	72.8	1 78
17	120	73.6	2.73
18	62	38.8	1 09
Mean ±S.D.	98.8 ± 22.6	59.2 ± 14.1	1.82 ± 0.52
19	72	44.2	2.0
20	140	83.8	2.2
21	113	66.4	1.86
22	142	86.1	2.94
23	121	68.0	1.69
Controls n = 15	127 9 ± 21.3	75.2 ± 10.3	1.93 ± 0.31

Table IV Degradation of albumin expressed in per cent of intravascular pool and in g/24 h. The kind of albumin used (Am. = Amersham, Abb. = Abbott) is stated in the right column

Case	Degradation rate in % of intravascular pool	Degradation rate in g/24 h	Degradation rate in g/kg/24 h	
Operable				
1	21.2	19.7	0.44	Am.
2	–	–	–	–
3	13.6	17.3	0.24	Abb.
4	11.5	10.5	0.18	Abb.
5	10.2	10.0	0.21	Abb.
6	10.5	8.8	0.14	Abb.
Inoperable without ascites				
7	27 0	38.6	0.61	Am.
8	16.0	14.7	0.36	Am.
9	19 1	21 4	0.34	Am.
10	3.6	3.5	0.09	Abb.
11	9.9	8.4	0.15	Abb.
12	9.2	14.1	0.20	Abb.
Inoperable with ascites				
13	14.8	17.2	0.26	Am.
14	25.2	22.9	0.44	Am.
15	18.1	16.1	0.32	Am.
16	10.4	12.0	0.19	Abb.
17	12.5	13.0	0.34	Abb.
18	15.1	9 4	0.17	Abb.
19	7.2	5.2	0.14	Abb.
20	7 4	10.4	0.16	Abb.
21	10.5	11.9	0.20	Am.
22	9.2	13.1	0.27	Abb.
23	18.8	22.8	0.32	Abb.

Degradation rate for Am. (Amersham) in controls 13.9 ± 3.29 %

Degradation rate for Abb. (Abbott) in controls 9.0 ± 0.99 %

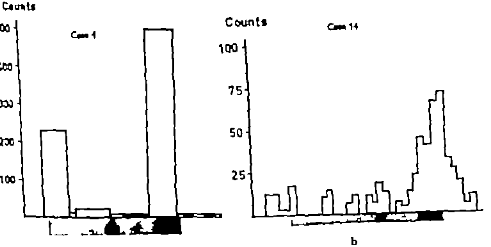


Fig. 2. The distribution of radioactivity in electropherogram of gastric juice from two cases of gastric cancer (cases 4 and 14). The maximum of radioactivity is in the albumin area. Some activity has more cathodic localization, probably derived from breakdown products from the albumin.

In the four cases in which the gastric juice was slightly blood-stained the amount of blood varied between 0.07 and 2 mm (see Methods) and, thus, it can be estimated that about 0.02 % of the activity in the gastric juice arose from plasma poured out with the bleeding

In all cases electrophoresis showed presence of albumin in the gastric juice, and the radioactivity was concentrated in the albumin-fraction. Fig 2 shows the electropherogram and distribution of radioactivity in two cases.

The precipitation procedure described under Methods was used for measuring the protein-bound radioactivity (TCA + PWA) free radioactivity present as ^{131}I-iodide ($AgNO_3$) and non-precipitable fraction, most probably ^{131}I-amino-acids (table V) Table V a also shows the values for a control group of normochylic and achylic patients (Wetterfors et al. 1960)

The highest values for protein-bound activity as well as for non-precipitable fraction, were obtained in the seven patients with achlorhydria in the other five patients with normochylia or moderate hypochylia the values accorded with those in the control cases.

In case 19 the gastrectomized patient in whom the radiograms were strongly suspected of showing evidence of cancer in the achlorhydric gastric remnant, only 1 % of protein-bound activity appeared in the gastric juice. Laparotomy showed later that cancer was absent from the gastric remnant.

In the six cases in which both the protein-bound and the non precipitable fraction excreted in the gastric juice (calculated per 60-minute samples) were determined as a percentage of intravascular activity the values showed a relatively great standard deviation (table V b) Transformation of the protein-bound and the non-precipitable activities into quantitative measures (g per 24 hours) gives varying values (approximately 2—6 g) which means that 1/4—1/2 of the amount of albumin catabolised per 24-hours in the respective cases was

Table V a. Protein-bound and non-precipitable radioactivity in gastric juice (expressed in % of total activity) in 13 investigated cases of gastric cancer

Case	Acidity	% protein bound activity (TCA + PWA)	% non-precipitable activity
1	An	8.7	17.8
4	An	48	27 6
5	An	18.2	3.8
7	An	31	6
8	An	16.5	—
13	An	23	—
14	An	37 7	34.3
19	An.0	1	—
Controls	An	6 ± 4.2	18 ± 4.5
2	HN	10.4	12.6
3	N	15	14 4
6	N	5.6	7.2
16	N	3.7	18.3
18	N	3.9	17 1
Controls	N	8.3 ± 4.9	11 1 ± 6.6

Table V b. Quantitative aspects on the excretion of radioactivity in the gastric juice

Case	Acidity	% of intravasc. activity excret./24 h	% prot. bound + non-prec. act. excret./24 h	Min. g alb. excret./24 h	Max. g alb. excret./24 h	% of total degrad./24 h via the stomach
2	HN	8.6	2.0	1 0	2.3	—
3	N	10.8	3.2	2.1	4 0	23
4	An	8.1	6 1	3.7	5 7	54
5	An	9.4	2.1	1 7	2.1	21
16	N	(24.6)	3.0	1 1	3.4	28
18	N	5.5	1.2	0.1	0.8	8.5

N = Normochylia.
HN = Hypochylia.
An = Achylia (achlorhydria)
0 = No cancer in the stomach.

Table VI. Elimination constants (K-values) for intravascular radioactivity and released into ... K_{iv}/K_{et} and $K_{iv}—K_{et}$ are also given. To the right the kind of ^{131}I-albumin used

Case	K_{iv}	K_{et}	K_{iv}/K_{et}	$K_{iv}—K_{et}$	
1	0.070	0.044	1.59	0.026	Am.
2	—	—	—	—	—
3	0.104	0 044	2.36	0.060	Abb.
4	0.040	0.031	1.29	0.009	Abb.
5	0.0[illegible]1	0.025	2.44	0.036	Abb.
6	0.075	0.048	1.56	0.027	Abb.
7	0.139	0.036	3.86	0.103	Am.
8	0.089	0.016	5.56	0.073	Am.
9	0.092	0.046	2.00	0.046	Am.
10	0.020	0.004	3.00	0.016	Abb.
11	0.053	0.032	1.66	0.021	Abb.
12	0 039	0.034	1.15	0.005	Abb.
13	0.058	0.025	2.32	0.033	Am.
14	0.063	0.024	2.63	0.039	Am.
15	0.127	0.057	2.23	0.070	Am.
16	0.039	0.029	3.07	0.060	Abb.
17	0.071	0.021	3.38	0.050	Abb.
18	0.057	0.011	5.18	0.046	Abb.
19	0.050	0.025	2.00	0.025	Abb.
20	0 035	0 024	1 46	0.011	Abb.
21	0.031	0.015	2.38	0.018	Am.
22	0.049	0.040	1.23	0.009	Abb.
23	0.099	0.064	1.55	0.035	Abb.

K_{iv}/K_{et} in controls Mean ± S. D
Am. = 1 76 ± 0.32.
Abb. = 1 49 ± 0.19.

excreted in the gastric juice. In an advanced case (case 18) with severe hypoalbuminaemia and ascites the leakage was very small, equalling 1/10 of the albumin catabolised in 24 hours. Only two of six investigated patients (cases 4 and 5) had achylia. One of these had the greatest leakage.

In case 14 the patient with almost complete pylorostenosis due to cancer in whom gastric drainage was maintained for 5 days, measurements of the

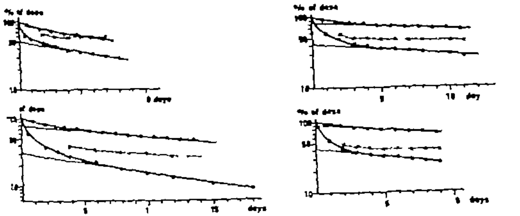


Fig. 3. Elimination of ^{131}I-albumin in four control cases. The two cases to the left represent ^{131}I-albumin, Amersham, and the two cases to the right RIHSA-M, Abbott. "Retained dose" is described by the upper curve and intravascular activity by the lower curve (in % of dose). The interrupted line is the "distribution curve" (see text).

fraction of intravascular activity excreted in 24-hour samples of retained gastric juice – on the presumption that the fraction of activity bound to protein was constant throughout the period of investigation — gave values of 8—13 % (TCA + PWA) and 15—24 % (TCA + PWA + non-precipitable fraction).

If the total amount of gastric juice is estimated as 1,500 ml per 24 hours, the value obtained for the mean 24-hour excretion of albumin will be a minimum of 40 and a maximum of 80 % of the albumin catabolized in 24 hours.

Loss of radioactivity in faeces

In the cancer patients the ^{131}I-albumin excretion in the faeces was less than 1 % of the injected dose throughout the period of investigation, that is, the same as in the controls.

Distribution of albumin

In the control group the quotient $\frac{K_{tr}}{K_{rt}}$ for the Amersham ^{131}I-albumin was 1.76 ± 0.52 and for the Abbott preparation 1.49 ± 0.19. This means that K_{tr} is even normally $> K_{rt}$, or in other words, that intravascularly activity falls more rapidly than does total retained activity (see Discussion) (fig. III).

$\frac{K_{tr}}{K_{rt}}$ could be calculated in 4 of the operable cases. It was normal in two being 1.57 (Am) 1.29 (Abb) and raised in the other two, being 2.56 and 2.44 (Abb) (fig. 4).

In the two inoperable groups (without and with ascites, fig. 5 and 6) the values for $\frac{K_{tr}}{K_{rt}}$ were generally elevated, with a few exceptions (table VI). In case 19 the value was raised, but normal in case 20.

Among the three patients investigated postoperatively the quotient $\frac{K_{tr}}{K_{rt}}$ was normal in two and slightly raised in one (table VI).

Another way of demonstrating equilibrium between intravascular and extra-

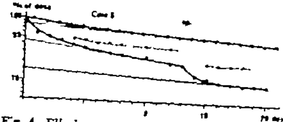


Fig 4 Elimination of ^{131}I albumin before and after operation in case 5 with resectable cancer of the stomach. The signification of the curves as in fig 3

Praeop. $K_{tv} = 0.061$ $K_{vt} = 0.025$ $\frac{K_{tv}}{K_{vt}} = 2.44$

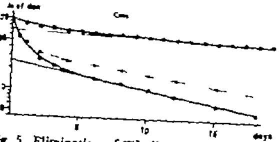


Fig 5 Elimination of ^{131}I-albumin in case 8 with inoperable gastric cancer without ascites. The distribution curve is falling and $\frac{K_{tv}}{K_{vt}}$ is high.

$K_{tv} = 0.089$ $K_{vt} = 0.016$ $\frac{K_{tv}}{K_{vt}} = 5.56$

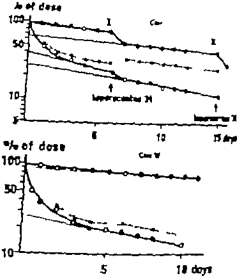


Fig 6 Elimination of ^{131}I-albumin in cases 16 and 17 with ascites. I the former laparocentesis was performed twice. The distribution curves are falling and $\frac{K_{tv}}{K_{vt}}$ high.

Case 16. I $K_{tv} = 0.089$ $K_{vt} = 0.029$ $\frac{K_{tv}}{K_{vt}} = 3.0$

II $K_{tv} = 0.058$ $K_{vt} = 0.026$ $\frac{K_{tv}}{K_{vt}} = 2.23$

Case 17 K_{tv} 0.071 $K_{vt} = 0.021$ $\frac{K_{tv}}{K_{vt}} = 3.38$

vascular pools is by plotting intravascular activity as a fraction of total activity ("distribution curve" Berson et al 1953) In the control group this distribution curve is practically horizontal, that is intravascular activity is a constant part of total activity (retained dose) (fig 3) and runs at a level of 40 ± 7 % This constancy is, on the whole also found in the operable group (fig 4) while in most cases in the inoperable groups the curve describes a declining course This means an increased extravascular distribution of ^{131}I albumin (fig 5 and 6) The effect of laparocentesis is also demonstrated (case 16 fig 6)

Dialysable radioactivity in plasma and ascitic fluid

In the control group (8 cases) the mean value for dialysable plasma-activity precipitable with $AgNO_3$ was 0.89 % and for the 6 gastric-cancer cases it was 0.94 %

Dialysis of ascitic fluid showed that in two patients (cases 18 and one case not recorded in other respects) the fraction of the activity precipitable with $AgNO_3$ contributed 0 9—1 4 % and in the third patient (case 19) 10.3 % of the total activity

Radioactivity in the stomach wall

No significantly increased uptake of radioactivity ^{131}I albumin or degradation products was registered in tumour tissue as compared with healthy gastric tissue, the mean value being 86 % (40—128) In those two cases, where comparison had to be made with jejunal tissue, the values were 115 and 127 %

Nitrogen-balance studies

In case 4 one of the operable cases, an eight-day study showed, as a total, a negative nitrogen balance of 14.9 g of N equivalent to 99.6 g of protein. Calorie intake was satisfactory and protein intake averaged 54 g per 24 hours. The faeces contained a normal amount of nitrogen, or 0.7—2.0 g per 24 hours. During this period of time no change occurred in the relative concentration of albumin in the blood.

Case 20, with stenosis of the cardia and malnutrition had for 10 days an overall negative nitrogen balance of 9.4 g of N equivalent to 63.3 g of protein. 24-hour samples of faeces contained up to 1.7 g of N (4 days). Over the same period of time the relative albumin concentration decreased slightly and then rose again. The plasma volume decreased by 120 ml during the 10 days covered by the study. This signified an almost unchanged level of intravascular albumin (140 g and 138 g respectively). Total protein decreased slightly from 244 g to 221 g.

Discussion

Hypoproteinaemia and hypoalbuminaemia

It has long been known that cancer of the stomach may often be accompanied by a relative hypoproteinaemia (see Petermann & Hogness 1948).

It is chiefly the relative albumin concentration that falls (Abels et al. 1942, Homburger & Young 1948), whereas α-globulins increase and β- and γ-globulins are largely unchanged (Petermann & Hogness 1948).

The results of the present investigation agree fully with those of earlier studies. Slight relative hypoproteinaemia was present in only six cases out of twenty. Relative hypoalbuminaemia was however noted in sixteen cases and was somewhat more marked in the inoperable cases. Accordingly relative hypoalbuminaemia seems to be the main disturbance whereas relative hypoproteinaemia is much less prominent.

The genesis of the relative hypoproteinaemia has been studied fairly attentively in recent years. To throw further light on this problem — from a quantitative aspect as well — the blood and plasma volumes in gastric cancer must first be considered.

Blood, plasma and red-cell volumes

In cancer of the stomach Fretheim (1954, 1955) has found that the blood volume is slightly reduced, mainly as a result of a reduced red-cell volume, but that the plasma volume is normal or only slightly reduced (referred to ideal weight). In cases of marked anaemia he reported a slightly increased plasma volume, probably a compensatory mechanism. Fretheim found that the more advanced the cancer the greater are the deviations from normal values. Rasmussen et al. (1944) observed that in patients with gastric cancer the total plasma volume was raised after a positive nitrogen balance for 2—3 weeks.

In inanition an unchanged or increased total plasma volume has been reported by Taylor et al. (1949). According to Hottinger et al. (1948) excessive starvation (body-weight reduced by 50 %) gives only a slight decrease of TBV.

To enable accurate assessment and comparison of TBV, PV and RCV as well as intravascular albumin, in a material of patients like that reported here without and with weight loss of a varying and sometimes ...

of magnitude, and to correlate the results with those in a control group suitable parameters are necessary. If only the absolute values for TBV, PV and RCV are compared, misleading results may be obtained, since other variables in the groups are not taken into consideration.

As regards the values for TBV, PV and RCV, "ideal weight" or body surface are given in the literature as representative parameters. But when large variations in body-weights occur the body-length seems to be the most suitable parameter (Gibson & Evans 1937, Perera 1946, Sjöstrand 1961).

The somewhat divergent mean body lengths in the analysed groups are not an important source of error, since there is a linear relationship between TBV and body length in the range concerned (> 150 cm) (Åstrand 1952).

In the entire material there is no significant change of TBV, PV or RCV either totally or referred to body length or body weight, compared with the controls. It thus seems as if gastric cancer generally has no direct influence on the TBV, PV or RCV.

Analysis of the material with respect to operability demonstrates a tendency to a lower RCV/m in the operable group. As these patients did not demonstrate a history of haemorrhage or rapidly growing cancer, this fact possibly suggests that RCV is the first blood-element to be influenced by the cancer.

In *the two groups of inoperable cases* normal values were obtained for TBV/m, PV/m and RCV/m. This suggests that extragastric extension of the cancer does not influence TBV or PV. The normal value for RCV/m compared with the former group may possibly indicate a restitution of the erythropoiesis of unknown mechanism. A tendency to lower-ing of PV/m in the group with ascites is certainly due to the development of ascites.

Stenosis of cardia with malnutrition and weight loss seems not to influence the TBV/m, PV/m or RCV/m.

In those cases where gastric resection had been made radically normal values were obtained, contrary to the not radically resected case (case 19) in which TBV/m, PV/m and RCV/m were very low.

Intravascular albumin

Up to now intravascular albumin has always been quoted in the literature as an absolute value or been referred to body weight. For reasons mentioned earlier, correlation with body-length is considered justified.

There is a clearly lower intravascular albumin pool in the cancer-cases. This fact becomes obvious when referred to body length, the fixed parameter. Per kg body weight, on the other hand, the values are normal.

In the *operable group* the total amount of intravascular albumin is moderately decreased, which is partly explained by the slightly lowered PV. Per metre of body length the absolute amount of albumin intravascularly is significantly lower than in the controls.

In the *inoperable group without ascites*, on the other hand, the amount of intravascular albumin is very moderately, not significantly lowered, despite a further decreased albumin concentration. This is probably explained by the tendency to a rise in PV that is seen in this group.

In the *inoperable group with ascites* the relative hypoalbuminaemia accounts for the low absolute values, partly attributable to the again decreasing PV. The significantly lowered absolute amount of

albumin does not influence the value per kg body-weight. Expressed as grams per metre the amount of intravascular albumin is clearly lowered, however

In the initial phase and as long as it is limited to the stomach, gastric cancer seems to give a moderate relative and absolute hypoalbuminaemia. The cause of the latter will be discussed later on. In the next phase, when the cancer spreads outside the stomach, the PV tend to increase in comparison with the previous phase, whereas the relative hypoalbuminaemia increases.

In the last stage with ascites, exudation occurs into a preformed cavity possibly as a link in the distribution anomaly discussed later on. The relative hypoalbuminaemia persists and the PV again tend to decrease. This gives an absolute hypoalbuminaemia.

According to Homburger & Young (1948) surgical removal of a gastric cancer would — in spite of a positive nitrogen balance — not lead to restitution of the relative albumin concentration. It seems that only palliative resection was performed in these authors cases.

Out of two totally gastrectomized patients (cases 21 and 22) who were investigated a long time after operation, one showed normal values after adequate care. The other patient had normal values, both relatively and absolutely The third totally gastrectomized patient was investigated too soon after operation to permit any definite conclusions to be drawn. These collected observations argue against the view that the hypoalbuminaemia must be irreversible in patients who have undergone a seemingly radical operation.

The significance of the influence of infusions of unlabelled albumin on the results obtained in the actual cases can be discussed.

Infusion of unlabelled albumin gives a lowered specific activity but does not interfere with the total amount of labelled albumin. An immediate extravascular transport of the "new" albumin takes place to restitute the proportions of pools. This possibly is attended with an accelerated transport of labelled albumin to the extravascular pool, with a consequently steeper slope of the plasma activity curve. With constant plasma volumes no other changes of the radioactivity in the blood will take place. Thus, in those cases where smaller amounts of albumin were given, no important changes occur and the catabolic values and the slope of the curves can be considered significant. In those cases where large amounts were given fluctuations of plasma volumes cannot be ruled out. This may give a change in the slope of the curve but the percentual value of the catabolism is unaltered.

Malnutrition and malabsorption

Among the investigated patients 5 had a history of malnutrition with weight losses of > 20 % of the original body weight.

Ariel et al. (1943) found in gastric cancer no correlation between the degree of relative hypoalbuminaemia and intake of food. Homburger & Young (1948) arrived at the same conclusion in patients who had undergone palliative resection for cancer. Taylor et al. (1949) in investigations by electrophoresis and the "Evans blue" method both before and after starvation leading to a weight-loss of 24 % found no or slight relative and absolute hypoalbuminaemia.

In two of our patients (cases 10 and 20) the cause was stenotic processes in the

cardia, with marked difficulty in oral nutrition

The patient (case 10) with inoperable recurrent cancer localized in the cardia had both a relative and an absolute hypoalbuminaemia (referred to body length) An especially interesting feature in this case is the extremely low albumin catabolism The same was seen though not so markedly in case 20 the other patient with stenosis of the cardia. The case shows that in spite of earlier malnutrition and a negative nitrogen balance during the period of investigation a constantly normal albumin concentration both relatively and absolutely is maintained Gitlin et al (1958) in an investigation of malnourished children with ^{131}I albumin found a normal T/2 suggesting normal catabolism. They considered that the hypoalbuminaemia in these children was not due to a raised rate of catabolism but was attributable to a reduced albumin synthesis as a result of liver damage

Among the rest of the patients case 1 with operable cancer had no relative but an absolute hypoalbuminaemia, while in cases 14 and 15 the patients had both relative and absolute albumin deficiency In the latter two cases there were however in addition leakage into the stomach and a pathological distribution

Malabsorption was not present as is indicated from the fact that a normal quantity of radioactivity was demonstrated in the faeces and that the nitrogen content in the faeces was normal A normal faecal excretion of nitrogen in gastric cancer has been reported earlier (Ariel et al. 1943 Jarnum & Schwartz 1960)

Accordingly on the basis of earlier studies and of the present investigation it can be stated that pure malnutrition with > 25 % loss of body weight alone seems not to give hypoalbuminaemia, either relative or absolute. If hypoalbuminaemia is present in cases of malnutrition, other factors, such as increased leakage into the stomach and pathological distribution of albumin, are concerned in the causation.

Reduced albumin synthesis

Restricted ability of the liver to synthesize albumin has also been much discussed as a possible cause of hypoalbuminaemia (Ariel et al. Homburger & Young 1948, Mider et al. 1950 Petermann 1960 and others)

In order to estimate the synthesis the incorporation of glycine into different plasma proteins has been studied both in man, with and without cancer and in animals. The results are conflicting (Ariel et al 1943 Ariel 1949 Norberg & Greenberg 1951)

At present the synthesis can be assessed only indirectly by studying changes in the relative albumin concentration in the blood in relation to the degradation, on the presumption that the plasma volume is constant. Since in most cases there did not occur during the relatively short period of investigation anything that could cause noteworthy fluctuation in the plasma volume, an estimation of the synthesizing capacity may be warranted in these cases.

In eight patients the albumin concentration was fairly constant being low in six of them. This means a rate of synthesis that merely compensates for the actual catabolism and also for the pathological distribution Expressed in grams, the values are normal or lowered. In two cases (cases 1 and 8) the synthesis is normal or slightly increased.

In two other patients (cases 5 and 12) on the other hand, the albumin con-

entration fell during the period of investigation despite a normal catabolic rate. In only one of these cases were there signs of a pathological distribution. Probably the albumin synthesis is reduced in these cases.

In another patient (case 11) an increase in the relative albumin content occurred, which indicates a synthesis exceeding the quantitatively lowered catabolism.

In gastric cancer the synthesis of albumin seems to be unchanged or to decrease sooner or later but when the hypoalbuminaemia has reached a certain level the synthesis suffices to compensate for the actual catabolism and distribution. The decrease seems to be independent of the presence of liver metastases.

In the majority of cases, then, a normal or reduced albumin synthesis plays a role by failing to balance the increased catabolism, but not by actually causing the hypoalbuminaemia.

In a few cases, however there seems to be a real increase in synthesis.

Albumin degradation

Direct measurements of the rate of albumin degradation in gastric cancer have not previously been made.

Birke et al. (1959) showed that the albumin degradation is increased in gastric cancer The work presented here confirms this observation.

On examination with ^{131}I-albumin of gastric-cancer patients there was, particularly in the inoperable cases, a retention of radioactivity (see p. 183)

That this retention is extravascular is evident from the divergence of the elimination curves describing intravascular activity and retained dose (see p 183) That there is no intravascular disproportion between protein-bound activity and nonprotein-bound activity in the cancer cases is shown by the fact, that the same values were achieved as in the controls if the serum were dialyzed.

Dialysis of ascites in two cases showed values for dialysable activity that agreed with those for serum. This indicates that eventual retention of degradation products is dependent not on any extravascular change in the ratio of protein-bound to nonprotein-bound activity but on the size of the extravascular space. This would mean that the percentage values will be the same as without retention.

At present very little is known about the mechanism that regulates albumin breakdown, except for the effect of certain hormones. Still less is known about this in the presence of pathological processes in the gastrointestinal tract.

It seems probable that at least in cases with gastrointestinal disorders followed by increased albumin leakage, the catabolism occurs on a percentual basis, i. e. a certain fraction of the intravascular albumin leaks every day This observation has been made recently (Engström, Ljungkvist, Persson & Wetterfors 1962) in a patient where the catabolic rate was independent of the amount of circulating albumin.

This means that eventually when the intravascular pool is low enough, the synthesis and the leakage are equal and thus the albumin concentration is constant, i. e. if the pathological distribution is not taken into account.

The percentual values is thus thought to be the unit most suitable as an expression of catabolism, but does not permit any consideration of the synthesis.

In the operable group the rate of catabolism is normal to moderately

raised, while in the inoperable cases it is clearly raised with a maximum in the group with ascites.

A normal albumin degradation was noted in some patients (cases 5 11 and 12), of whom one (case 12) exhibited neither relative nor absolute hypoalbuminaemia and had normal distribution It may be mentioned that this patient was the youngest in the series (32 years)

It has been shown that in these studies the albumin catabolism was, in general moderately to distinctly increased but as regards periodicity or other factors influencing the rate of catabolism nothing is known. Such factors in combination with reduced synthesis may be a cause of hypoalbuminaemia in some cases.

The raised rate of catabolism is probably attributable to several causes. One is the increased leakage of albumin into the stomach Another possible cause could be a pathological protein metabolism in the cancer cells. These aspects will be discussed later

As mentioned earlier Gitlin et al. found a normal albumin catabolism in malnourished children This finding was of interest in relation to our observations, namely the lowered or subnormal catabolism in cases of malnutrition as in cases 10 and 20 with high stenosis. Case 10 illustrates the inoperable condition with both relative and absolute hypoalbuminaemia, while the patient (case 20) with operable stenotic cancer has normal albumin pools and normal distribution.

Quite the contrary is seen in patients with low stenosis in the stomach, who have a distinctly increased albumin breakdown (case 14) This will be discussed later

Leakage of albumin into the stomach

Schwartz & Jarnum (1959) found an increased faecal excretion of [131]I-labelled polyvinylpyrrolidone in three patients with gastric cancer

Using [131]I-albumin Birke et al. (1959) showed that there is an increased leakage of albumin in gastric cancer Paper-electrophoretic studies also revealed the presence of albumin. The latter observation was later verified by Jarnum & Schwartz (1960) and Glass & Ishimori (1961) By quantitative paper-electrophoresis of gastric juice from achylic patients with gastric cancer the last-named authors were able to demonstrate albumin in amounts varying between 20 and 500 mg per 100 ml.

In our achlorhydric patients with gastric cancer the protein-bound activity and in some cases the non-precipitable activity too in the gastric juice clearly exceeded that in achlorhydric patients without cancer In the normochylic and hypochylic patients values close to normal were obtained in spite of buffering If in the latter cases the low protein-bound activity were dependent upon an intragastric degradation, the non-precipitable fraction of the activity should be increased. Since it was not the observed leakage should in the normochylic cases of gastric cancer be regarded as normal. Glass & Ishimori reached the same conclusion from electrophoretic studies.

At present it seems, therefore, that a pathological leakage of albumin occurs particularly in cases of gastric cancer with achlorhydria, whereas in patients without achlorhydria, examined with current methods, no definite pathological features are revealed.

Some observations in this respect were made in the patient (case 19) in whom the radiological findings strongly pointed

to cancer in the achlorhydric gastric remnant. Only 1 % of the excreted activity was protein-bound. Laparotomy showed that cancer was not present. This lends further support to what has been suggested earlier namely that it is the cancer (with achlorhydria) and not the achlorhydria that causes the pathological leakage.

The fraction of intravascular activity excreted in the gastric juice per 24 hours (calculated per 60 minute sample) varied, but was slightly increased compared with a normal material (Wetterfors et al.) The protein-bound fraction was highest in the two patients with achlorhydria. A quantitative calculation of the size of the leakage and correlation with albumin degradation under the reported experimental conditions show that in four of the cases 1/4 to 1/2 of the albumin catabolized in 24 hours leaked out via the stomach. Cancer with pylorostenosis and retention gives a greatly increased leakage accounting for the greater part of the increased catabolism.

A comparison between this case of stenosis distally and the other cases leads to the conclusion that the 60-minute collections of gastric juice and the values hence derived are merely semiquantitative and represent an absolute minimum for the albumin leakage. The high relative quantity of protein-bound activity is, however indisputable.

The question of the physiological leakage in the small intestine in gastric cancer has not yet been studied.

The possibility of a pathological protein metabolism in cancer cells must also be considered. Studies by Babson & Winnick (1954) Busch & Greene (1955) and Busch et al. (1956) have shown that certain experimental tumours in rats accumulate and utilize plasmaproteins, especially albumin. Whether these observations are applicable to cancer in man is not clear

A direct uptake of ^{131}I-albumin, or radioactive degradation products in the tumour tissue does not seem to occur on comparison with healthy gastric tissue. This, in itself, is not inconsistent with the possibility *that the cancer cell* can catabolize ^{131}I-albumin, after which the degradation products are excreted. This question will have to be left open for the present.

Distribution of albumin

As mentioned in the foregoing, certain criteria must be satisfied before a correct quantitative estimation of the distribution of albumin in the body can be obtained. These criteria are a constant equilibrium between intravascular and extravascular pools, irrespective of their absolute size, and the quickest possible excretion of the degradation products (Sterling, Berson et al. McFarlane and others)

As was recently shown by Lewallen et al. (1959) there is no exact parallelism between intravascular activity and retained dose. They supposed an extravascular compartment with very slow exchange with the intravascular compartment.

If $\frac{K_{iv}}{K_{a}}$ in Lewallen's two cases is calculated, about the same values as in our control cases will be obtained. Among the operable cancer-cases there is in two cases an increase of the quotient $\frac{k_{iv}}{k_{n}}$. This increase is very marked in the inoperable cases. There are several possible explanations of this extravascular retention of radioactivity

One explanation is a real increase in the distribution of [131]I-albumin extravascularly in one or several compartments, *from which* a very moderate redistribution to the circulating blood occurs.

True retention of [131]I-albumin and albumin occurs in patients with ascites. That the ascitic fluid contains a very large part of the extravascular albumin is evident from, for instance case 16 in which the influence of the laparocentesis on the extravascular pool is clearly seen

In the inoperable cases without ascites an increased $\frac{K_{Iv}}{K_{ri}}$ is likewise obtained This suggests that the extravascular retention of [131]I-albumin is not conditioned by active local accumulation of exudate alone, but is a systemic disturbance, probably due to the cancer and particularly associated with extragastric growth.

The fact that the radioactive uptake in the tumour tissue is not higher than in healthy tissue argues against local retention in the tumour

Analysis of the elimination curves for [131]I albumin shows that equilibrium never exists between intravascular and extra vascular activity during the actual periods of investigation The extravascular activity does in fact reach a maximum after 5—6 days and then falls slowly but the fact that this slope is less steep than the intravascular one implies that the distribution rate I V→E. V is higher than E V→I V This is also mirrored in the falling distribution curve Accordingly the slope of the plasma-activity curve expresses the combined effect of catabolism and further continuous distribution to the extravascular compartment. For this reason it has not been possible to calculate in the usual way the size of the extravascular pool.

An increase of the extravascular compartment and a rise of the albumin concentration extravascularly are the two possibilities that might explain the observations made.

Ascites represents both these possibilities. In cancer-patients ascites is the result of an exudative process which occurs independently of the actual blood-albumin level. In all probability there occurs an analogous process in the patients without ascites, but in them it is manifested as not yet defined extravascular compartments. Since [131]I-albumin here reflects the kinetics of albumin these results indicate a large extravascular albumin pool, which is not constant in its relation to the intravascular pool This lack of constancy and equilibrium is most probably one of the main causes of the relative and absolute hypoalbuminaemia in cancer The explanation of this extravascular retention seems to be a general disturbance of permeability which is most marked in the inoperable cases

Summary and conclusions

Relative hypoalbuminaemia is the most prominent feature of the serum-protein pattern in gastric cancer It appears irrespective of the relative total protein concentration. The relative hypoalbuminaemia tends to increase with the spread of the cancer

The ideal parameter in the comparison of TBV PV RCV and intravascular albumin is body length whereas body weight seems to be unsuitable, because of the individual variations in weight loss

TBV/m, PV/m and RCV/m in a mixed material of gastric-cancer patients are unchanged in comparison with a control group

Progress of cancer with or without inanition gives no significant changes in the TBV correlated to body-length, the value remaining normal throughout the course of the disease. The PV undergoes minor changes, while the RCV is at first lowered and later becomes normal.

Cancer of the stomach is accompanied by a reduced amount of albumin intravascularly both totally and calculated per m body-length.

Albumin catabolism expressed as a percentage of the intravascular pool is *moderately to distinctly increased.*

The increased degradation rate is in part attributable to an increased leakage of albumin into the stomach. This leakage is not referable to any bleeding from the cancer. The leakage is greatest in cancer-cases with achlorhydria, whereas the normochylic cancer-cases show a normal or slightly increased leakage.

Calculated in terms of quantity or correlated with the catabolic rate the leakage shows varying values, according to the presence or absence of achlorhydria. With distal stenosis and retention the greater part of the increased catabolism is occasionally referable to the leakage into the stomach.

The hypoalbuminaemia seems partly to be caused by a systemic disturbance of permeability which is most marked in the inoperable cases and manifests itself by an increased extravascular distribution of albumin. A final stage of this process is the development of ascites. There seems not to be any demonstrable steady state in these cases.

High stenosis with malnutrition is accompanied by a low catabolic rate. The relative and the absolute albumin concentrations are maintained. Hypoalbuminaemia in such cases is most probably due to a reduced synthesis in the liver or a pathological distribution.

Malnutrition per se, therefore, is very unlikely to be a causative factor of the hypoalbuminaemia in gastric cancer.

Malabsorption does not seem to be concerned in the causation of hypoalbuminaemia in gastric cancer.

Patients who have earlier undergone radical operation for gastric cancer have *neither relative nor absolute* hypoalbuminaemia. Degradation and distribution of albumin are normal in these cases.

As judged empirically albumin synthesis is most likely normal or decreased in gastric cancer. In some cases, however, the synthesis may be increased.

Acknowledgement

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Continued Studies on Myocardial Infarction in Malmö

Our 1935 to 1954 Material Completed for the Years 1955 to 1959

By

J. Sievers and G. Blomqvist

Based on a material of 1 612 patients, hospitalized during the years 1935—54 for acute myocardial infarction in Malmö, Sweden, we have, in a number of earlier publications (1—6) presented some epidemiological, clinical and prognostic considerations as to the mentioned condition. Though a more comprehensive study on this subject is under preparation (7) we have found it feasible already now to present some findings concerning the cases of acute myocardial infarction hospitalized in our clinic during the subsequent 5 year period of 1955—59.

As has been pointed out in our earlier publications, Malmö is a town of now about 220,000 inhabitants with good opportunities for epidemiological research since the town has only one large university hospital and no private ones. The patient material in the hospital is solely derived from the town. Furthermore the autopsy frequency at the hospital is 92.5 % which gives good opportunities to check the accuracy of the clinical diagnoses.

Submitted for publication January 15, 1962.

During the years 1935—54 a total number of 1,821 acute myocardial infarctions were hospitalized. Of these 1,530 were primary infarcts, as estimated from the hospital records, 238 second infarcts, 47 third infarcts, 5 fourth infarcts while 1 patient had at least five infarctions. We have now undertaken to go through the hospital record material from the years 1955—59 incl. to follow up the trends found in the earlier 20 year material. During the last 5 year period another 1,067 cases of acute myocardial infarction were hospitalized. Of these 936 were primary infarcts, 120 second infarcts and 11 third infarcts.

A short summary will be given as to the criteria used for the diagnosis of acute myocardial infarction. For further details see one of our previous papers (1).

These criteria have been:

1. The observation at autopsy either of a fresh myocardial infarction or of a fresh coronary occlusion in the presence of typical and acute clinical symptoms.

2. Typical electrocardiographical pattern either alone or — more often — together with a typical case history and typical laboratory data.

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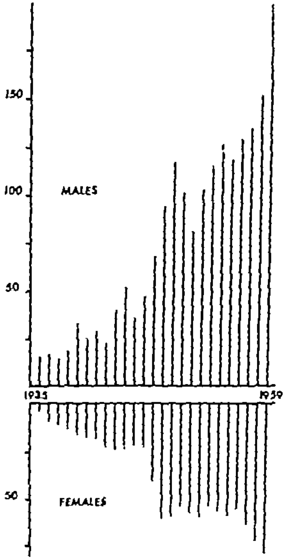


Fig 1 No. of cases by sex and years 1935—1959
—— First inf.
Second inf.
---- Additional inf.

3. In the absence of typical electrocardiographical changes we have accepted cases with a typical clinical case history typical clinical picture and laboratory data consistent with the diagnosis of myocardial infarction.

The *frequency* per calendar year during the entire 25 year period in males and females is given in fig 1 Before the figures for the last five years were available we have in earlier papers discussed the possibility of a rather steep increase in

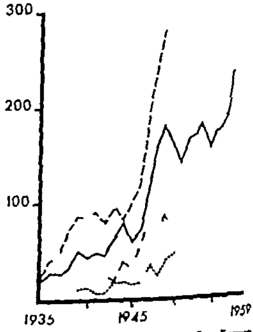


Fig 2. No. of cases per year in some Scandinavian materials.
—— Ullevål (Oslo, Norway)
---- Ekvall (Umeå, Sweden)
— — Lindén (Uppsala, Sweden)
—— Own material (Malmö, Sweden)

the frequency during the late forties and we have thought that our material gave basis for the assumption that a reasonably steady state was reached in the year 1949 Fig 1 reveals that this assumption was not too accurate. Apparently a continuous increase has prevailed during the entire 25 year period as to the absolute numbers of acute infarctions hospitalized.

Fig 2 is once again a figure from an earlier paper (1) where the last 5 year figures are added. Until the late forties the increase of primary infarctions in our material seems to follow that of the Ullevål-material (8) in Oslo fairly well, whereas after that no information is available for the Oslo material.

The mean *sex ratio* was 1 66 males versus 1 female in the 20 year material 1935—54 and has shown a slight increase during 1955—59 being on an average

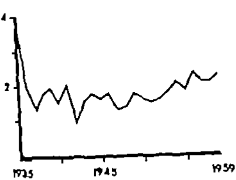


Fig. 3. Sex ratios for the years 1935—1959.

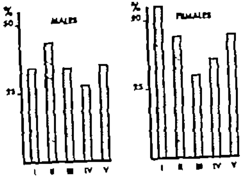


Fig. 5. Age adjusted 4 weeks mortality rates.
I = 1935—39.
II = 1940—44.
III = 1945—49.
IV = 1950—54
V = 1955—59



Fig. 4. Average age
—— Males.
---- Females.

2.09 1 Fig 3 gives the sex ratios for the entire 25 year material. The figure for the first year 1935 is based on very few cases and has to be considered a chance variation. Our figures correspond fairly well with those found in ther Scandinavian series where sex ratios be tween 1.2 1 and 2.3 1 are found (9 10, 11 12 13, 14 15)

The mean age for the primary infarc tion also shows for the last 5 year period good continuity when compared with the earlier 20 year material. As can be seen from fig 4 there has been, during the entire 25 year period, continuous increase in the mean age as well for males as for females. During the last 5 year period there has been an overall mean age for males of 63 9 with slowly increasing tendency Among th females the overall mean age during the last 5 year period was 69 3 Also here a certain increase per calendar year was noted.

The *acute mortality* during the hospitalization for primary infarctions during 1955—59 was 31.3 % as compared with 35 7 for the previous 20 year material. The corresponding figures for females were 42.6 and 38.1 respecti ely Since the acut mortality correlates positively with age t infarction and since the mean age has changed slightly these figures are not quite comparable. In fig 5 the mortality rates are therefore presented, corrected for the discrepancy as to age composition.

Figures for acute mortality vary considerably in the literature, most certainly in many cases due to differences as to composition of the material and comparisons with other investigations are here even more hazardous than usual However our figures seem to be of about the same magnitude as those in some

Table I Severity groups according to Helander Frequency distribution in five-year periods

Years	Group I Severe %	Group II Medium %	Group III Mild %	No. of patients
1935—39	19.8	79 0	1.2	86
1940—44	15.5	79.5	5 0	181
1945—49	18.8	76.2	5.0	357
1950—54	18.9	75.8	4.3	532
1955—59	19 1	76.8	4.1	844

other Scandinavian materials (e. g 15) while others find higher acute mortality (e. g 11 16)

For *severity grouping* we have been using throughout a classification first put for ward by Helander (10) The grouping system consists of 3 groups, group I severe cases, group II medium cases and group III mild cases. For details concerning grouping criteria the reader is referred to Helander's original paper

As can be seen from table I we had during the first 20 year period a fairly uniform distribution of our cases in the 3 different groups. In this last 5 year period also this distribution corresponds fairly well not to say extremely well, with the previous figures. Thus 19 1 % was assigned to group I severe cases, while 76.8 % was assigned to group II, medium cases, and finally 4 1 % to group III mild cases.

We find it important to stress the fact that there has been no substantial change in the severity distribution with calendar year in this investigation. One of the major objections in the literature to the "reality of the frequency increase of myocardial infarction is that the diagnosis of the condition was not made during earlier years and that the increase found in many countries throughout the civilized world is only a result of more correct diagnoses. In our opinion the present material gives us reason to believe that this assumption does not hold true for Malmö. If it were so, we would have found an increasing frequency of milder infarctions during later periods. Table I shows that this is by no means the fact.

The percentage of cases in each severity group as well as the mortality within 4 weeks in these groups are given in table II, together with the corresponding figures from some other Swedish materials.

Table II Severity groups according to Helander Frequency distribution and mortality in some Swedish materials

	Group I (severe)		Group II (medium)		Group III (mild)		No. of patients
	% of all cases	Mortality %	% of all cases	Mortality %	% of all cases	Mortality %	
Helander (1950) (10)	29 0	57	60.1	22.4	10.9	0	193
Wållgren (1950) (12)	20.9	62.5	75.2	23.5	3.9	0	163
Lindén (1952) (13)	43	71	42	35	15	6.5	512
Helander & Levander (1959) (15)	23.8	54	68.2	27	8.0	4	286
Own material 1935—54 (1)	18.9	79.8	76.7	28.3	4.4	2.0	1 156
Present material 1955—59	19.1	85.7	76.8	22.7	4.1	0	844

Table III. Transaminase values

Severity group according to Helander	Mean value ± S.E.	No. of observations
Severe	202.7 ± 42.4	35
Medium	119.3 ± 5.5	299
Mild	67.0 ± 14.8	15

Another obvious reason for the found increase in exact figures would be the increase of the population in Malmö. In an earlier paper (1) we have presented a figure giving the increase for males and females both in separate age groups and with all age groups taken together. The figures were given as cases of myocardial infarction per 1 000 inhabitants in corresponding age groups. Fig. 6 presents the same picture with the curves extended for the last 5 year period. Apparently the increase prevails even when the age composition and the increase in total number of inhabitants in Malmö is taken into consideration.

The *glutamic-oxalacetic transaminase* (GOT) in cases of myocardial infarction has been studied by a large number of investigators, among others by Hanson and Biörck at this Department (17, 18). For detailed literature references in this connection the reader is referred to the extensive literature survey by Wróblewski (19).

In the present material the GOT in serum was determined in 56.7 % of the material on the second, third (or both) day after clinical onset. In table III the mean values and the standard errors are given for the 3 severity groups. A statistically significant correlation appears between the GOT mean values and the severity as judged by the Helander grouping criteria. This was to be expected, since a correlation between the extent of the infarction and the GOT levels has been shown by a number of investigators.

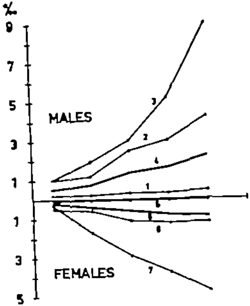


Fig. 6. No. of hospitalized cases of myocardial infarction per 1,000 inhabitants by sex and age groups, Malmö 1935—1959.

1) Males 30—49		5) Females 30—49.	
2)	50—69.	6)	50—69
3)	> 69.	7)	> 69.
4)	total.	8)	total.

Finally some figures concerning the frequency of *myocardial rupture* might be of interest.

The total number of deaths 1955—59 was 373 (224 males and 149 females). 94.2 % of the males and 89.9 % of the females were autopsied, which gives a total autopsy rate of 92.5 %.

Thirty-five ruptures were observed in 345 autopsies, giving a rupture frequency of 10.1 %. In table IV the percentage of ruptures in different age groups for males and females is given.

Table IV Myocardial ruptures found at autopsy

Age	Males		Females		Total	
	Ruptures %	No. of autopsies	Ruptures %	No. of autopsies	Ruptures %	No. of autopsies
30—49	0.0	8	0.0	3	0.0	11
50—69	9.5	116	5.9	51	8.4	167
0—89	4 8	84	20.0	80	12.2	164
90 and >	33.3	3	—	—	33.3	3
Total	7 6	11	14.2	134	10.1	345

In our previous 20 year material the overall frequency of rupture was slight ly higher 13 0 % The increasing frequency with age, obvious in the present material was also noted in our previous series and is well in agreement with the findings of other investigators (e. g 20)

A higher incidence of rupture among females than among males is reported previously This is also the fact in the present material, the age adjusted sex difference being highly significant (p = 0 001) No definite explanation to this difference is agreed upon in the liter ature, though some suggestions as to the mechanism have been put forward (e. g 20)

Summary

As a complement to a previously re ported 20 year material of 1 821 hospitalized cases of myocardial infarction the following 5 year period of 1955—59 is reviewed and an additional material of 1 067 cases is presented, bringing our total 25 year material to a number of 2 888 cases.

The present paper is a preliminary report only giving figures on a selected number of items. A more extensive study of the material is under preparation.

The continuous increase with calendar year both in absolute figures and cor related to a well defined base populatio is presented and the distribution with severity groups during different tim periods is discussed.

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Figures on the glutamic-oxaloace transaminase content of serum are presented and the positive correlation between these and the severity of the infarction as judged clinically is demonstrated.

Finally figures are given on myocardial rupture A frequency of 10 1 % is found in our autopsy material The earlier known increase with age is demonstrated as is the statistically higher rupture frequency among females than among males.

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Table IV Myocardial ruptures found at autopsy

Age	Males		Females		Total	
	Ruptures %	No. of autopsies	Ruptures %	No. of autopsies	Ruptures %	No. of autopsies
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50—69	9.5	116	5 9	51	8.4	167
70—89	4.8	84	20.0	80	12.2	164
90 and >	33.3	3	—	—	33.3	3
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In our previous 20 year material the overall frequency of rupture was slightly higher 13 0 %. The increasing frequency with age, obvious in the present material, was also noted in our previous series and is well in agreement with the findings of other investigators (e. g 20)

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Myeloma and Pernicious Anaemia

By

Sten Olle Larsson

It is known that hypogammaglobulinaemia may occur in association with pernicious anaemia (12). Since the level of the normal gammaglobulin is low also in myeloma it was considered worth while investigating the frequency of pernicious anaemia in patients with myeloma.

Myeloma is nearly always accompanied by some degree of anaemia (1, 2, 9, 22). According to Osserman (18) the development of anaemia in myeloma may be due to one or more of several factors, such as depression of the red bone marrow, hyperhaemolysis, blood loss, renal insufficiency, chemotherapy and radiotherapy, complicating infections and nutritional factors. Though megaloblasts have occasionally been found in the bone marrow (7, 15, 20), judging from the literature fullblown pernicious anaemia appears to be rare in myeloma. In 1956 Mandema (15) described myeloma in a patient with known pernicious anaemia. Two instances of co-existing myeloma and pernicious anaemia in one and the same family have been reported. Mandema and Wildervanck (16) thus described 3 sisters, 2 of whom had myeloma and the third pernicious anaemia and Herrell et al. (8) reported myeloma in 2 brothers, whose mother had died from pernicious anaemia.

Material and methods

The material consisted of all patients, who had been admitted to the Medical Department, Malmö General Hospital, in 1951—1960 because of myeloma. Of the 69 patients thus traced, 38 were females and 31 males. In 5 of these patients pernicious anaemia had also been diagnosed. In 1 of them (No. 1 in table I) who had died before the routine clinical use of the Schilling test, the diagnosis had been made on the basis of the usual findings, i.e. hyperchromic anaemia, histamine refractory achlorhydria and satisfactory response to B_{12}-treatment. In 3 cases (Nos. 2, 3 and 4) the B_{12}-absorption test confirmed the existence of an intrinsic factor-deficiency. In the fifth case the inability to absorb vitamin B_{12} might have been of some origin other than intrinsic factor deficiency.

A diagnosis of pernicious anaemia had also been made in 2 cases of "narrow band" or benign monoclonal hyperglobulinaemia, i.e. cases with a pathological globulin component without roentgenological changes or evidence of myeloma in the bone marrow (Nos. 6 and 7) (23). These cases were not included in the myeloma series.

In addition, 2 cases seen at another hospital were placed at my disposal. One (No. 9) of

Submitted for publication January 15, 1962.

Table I Laboratory findings in the cases described

Case	Year of birth	Sex	Hb g/100 ml	Erythr mill/mm³	Serum B_{12} conc. μμg/ml	Schilling test Urin. excr % in 24 hrs Without intrinsic factor	With intrinsic factor	Bone marrow	Gastr. secr.	ESR mm/1 hr	Total
1	1871	♀	10.2	2.3	—	—	—	Plasmac.	Achlorh.	96	8.9
2	1889	♀	10 7	2 6	6	2.0	14	Plasmac. megalobl.	Achlorh.	70	8.8
3	1883	♂	13 7	3 7	—	2.0	5 (9)	Plasmac.	—	33	6.3
4	1875	♀	11.5	3.5	34	7 0	22	Plasmac.	Achlorh.	62	8.7
5	1887	♀	4.2	1 0	—	0 8	3	Plasmac.	Achlorh.	97	8.4
6	1900	♂	8.6	1 7	40	—	—	Megalobl.	Achlorh.	31	8.3
7	1882	♀	12.1	3.3	—	7.0	13	Normal	Achlorh.	31	8.4
8	1892	♀	10.5	3 1	—	2.7	—	Normal	Achlorh.	47	9.5
9	1889	♀	3 7	1.0	25	7 4	—	Plasmac. megalobl.	Achlorh.	143	7.3

The values refer to the time of diagnosis of pernicious anaemia which was settled before the protein disorder was found.

During treatment with vitamin B_{12}, N original values of Hb and erythrocytes at the time of diagnosis of pernicious anaemia were obtained.

the cases was a typical example of myeloma, while the other (No. 8) must be provisionally classified under the heading of benign monoclonal hyperglobulinaemia. Both showed the typical clinical picture of pernicious anaemia.

The serum B_{12} concentration was determined microbiologically with the use of Euglena gracilis strain Z (≲ 100 μμg/ml considered as low (10)). The vitamin B_{12} absorption test was performed according to Schilling (21) with an oral dose of 0.5 μg $B_{12}Co^{60}$. The serum proteins were analyzed by paper electrophoresis with the method of Laurell et al. (13). Immuno-electrophoresis according to Grabar and Williams was used (4).

Case reports

Case 1 A woman, born in 1871 who had always felt well, was admitted to hospital for the first time on Dec. 12, 1949 because of cerebral thrombosis. On admission she had hyperchromic anaemia. Hb 10.2 g/100 ml and erythrocytes 2.3 mill./mm³ Fe 76 μg/100 ml ESR 76 mm/1 hour. Total serum protein 7 8, globulin 2.8 g/100 ml. The

Serum protein pattern g/100 ml								Therap. effect of vit. B_{12}	Immunoel. phor type	Diagnosis
Alb.	α_1-glob.	α_2-glob.	glob.	β-glob.	γ-glob.	Abnorm. comp. Conc.	Abnorm. comp. Mobility			
2.47	0.66		1.03		4.71		γ	Good	—	Myeloma + pernicious anaemia (?)
4.17	0.58	0.72	0.55	0.32	0.59	2.08	γ	Good	γ	Myeloma + pernicious anaemia
3.9	0.49	0.71	0.56	0.25	0.58	All excreted in urine	γ	Initially good	γ	Myeloma + pernicious anaemia
3.96	0.38	0.64	0.43	0.26	3.01		γ	No	γ	Myeloma + pernicious anaemia
2.95	0.41	0.61	0.41	0.51	0.50	3.23	γ	Initially good	γ	Myeloma + malabsorption syndrome
4.14	0.37	0.60	0.60	1.60	1.18			Good	γM	Benign monoclonal hyperglob. + pernicious anaemia (?)
4.36	0.47	0.69	0.66	0.89	1.34		β_1—β_2	Doubtful		Benign monoclonal hyperglob. + pernicious anaemia
4.86	0.46	0.71	0.57	0.36	0.84	1.71	γ	Initially good	γ	Benign monoclonal hyperglob. + pernicious anaemia (?)
3.32	0.47	0.57	0.41	0.31	0.40	1.82	γ	Good	γ	Myeloma + pernicious anaemia (?)

With double dose of intrinsic factor
The concentration of the abnormal component is included in the underlined globulin values.

formol-gel test was positive after 2 1/2 hours. The sternal bone marrow contained numerous mononuclear cells with abundant cytoplasm, resembling plasma cells. Myeloma was suspected. Treatment with iron, liver extract and later also with folic acid produced no increase in the number of reticulocytes, but the Hb and the erythrocyte count rose and on Feb. 6, 1950 the Hb was 12.3 g/100 ml and the erythrocytes 3.9 mill./mm³ On April, 19 1951 roentgen examination revealed osteolytic lesions suggestive of myeloma. The diagnosis of myeloma was now regarded as definite.

On re-admission in May 1951 the Hb was 9.9 g/100 ml, erythrocytes 2.9 mill./mm³ ESR 75 mm/1 hour Total protein 8.1 globulin 5.6 g/100 ml. The formol-gel test was positive after 20 minutes. Bence-Jones protein was found in the urine. Since the cause of the anaemia was obscure only expectant treatment was decided upon. The following year the blood values fell, and in June 1952 the Hb was 8.1 g/100 ml, erythrocytes 1.5 mill./mm³ and the ESR 90 mm/1 hour The patient was admitted again because of her progressive anaemia. The sternal bone marrow still showed numerous plasma cells, but no megaloblasts. Price Jones curve showed shift to the right. Test meal hista-

mine refractory achlorhydria. Serum protein electrophoresis: total protein 8.9, albumin 2.47, α-globulin 0.66, β-globulin 1.05, γ-globulin 4.71 g/100 ml. Treatment with urethane was started on July 31, 1952 and on Aug. 15 also vitamin B_{12} injections were added. This treatment produced a considerable objective improvement. On Oct. 20, 1952 the Hb was 11.3 g/100 ml, erythrocytes 3.5 mill./mm³ and on Jan. 26, 1953 the Hb was 12.3 g/100 ml and the erythrocytes 3.9 mill./mm³. The patient was then given 100 μg vitamin B_{12} every 14th day. In the autumn of 1953 the patient complained of increasing back pain, and on re-admission in Oct. 1953 roentgen examination revealed compression of the 5th and 8th thoracic vertebrae. Hb was now 10.2 g/100 ml, erythrocytes 3.3 mill./mm³, ESR 97 mm/1 hour. The patient gradually became worse and died on Dec. 4, 1953.

Post mortem examination showed myeloma. The typical roentgen findings confirmed the diagnosis of myeloma. That B_{12}-deficiency was a contributory cause of her hyperchromic anaemia was evident from the good response to vitamin B_{12}-therapy. The histamine refractory achlorhydria argued for pernicious anaemia, but the B_{12}-deficiency might also have been due to some other disorder of the vitamin B_{12}-absorption. The poor response of the anaemia to therapy in the later course was probably due to progress of the malignant basic disease.

Case 2. A housewife, born in 1889, in whom achlorhydria had been diagnosed 20 years previously, was admitted on Nov. 5, 1956 because of cholecystopathy. Physical examination revealed nothing of interest except slight tenderness below the right costal arch. Hb 11.5 g/100 ml, erythrocytes 3.3 mill./mm³, leucocytes 3 500/mm³, ESR 100 mm/1 hour. Serum protein electrophoresis: Total protein 7.7, albumin 3.58, α_1-globulin 0.44, α_2-globulin 0.75, β_1-globulin 0.42, β_2-globulin 0.33, γ-globulin including abnormal γ_1 fraction 2.17 g/100 ml. Roentgen examination of the skeleton revealed no signs of a pathological condition. The sternal bone marrow contained a slightly increased number of plasma cells. During the following 2 years she was troubled by paraesthesiae of the hands and feet. On re-admission on Sept. 16, 1958 the tongue was red and smooth. Hb 10.7 g/100 ml, erythrocytes 2.6 mill./mm³, leucocytes 2 400/mm³. Platelets 170,000/mm³. Reticulocytes 34,000/mm³. ESR 70 mm/1 hour. Serum vitamin B_{12} 6 μμg/ml. The Schilling test without and with intrinsic factor showed an excretion of 2 and 14 % in 24 hours. Serum protein electrophoresis: Total protein 8.8, albumin 4.17, α_1-globulin 0.38, α_2-globulin 0.72, β_1-globulin 0.33, β_2-globulin 0.32, γ-globulin 0.59, abnormal γ_1 fraction 2.08 g/100 ml.

The sternal bone marrow now showed a few megaloblasts and an increased number of plasma cells, 144/1 000 nucleated cells. Roentgen examination still showed no signs of myeloma. The patient was treated regularly with vitamin B_{12} parenterally during follow up at the outpatient department.

The patient was last examined in 1961. There were no signs of bone destruction in skull, pelvis, thorax or vertebral column. No bone pains. No anaemia. No further increase in pathological globulins. The diagnosis of myeloma is therefore not definitely established and it is possible that the diagnosis should be essential monoclonal hyperglobulinaemia. The Schilling test demonstrated intrinsic factor deficiency as the cause of the low serum vitamin B_{12}-level.

Case 3. The patient was a retired tinsmith, born in 1883. In 1956 pernicious anaemia had been diagnosed by his family doctor who had since treated him regularly with vitamin B_{12} injections. Owing to progression of existing eczema, treatment with Triamcinolone was started in July 1959. During the summer of 1960 the patient complained of low back pain. He was admitted on Aug. 23, 1960. On admission Hb 13.7 g/100 ml and erythrocytes 3.7 mill./mm³. ESR 35 mm/1 hour. No proteinuria. N.P.N. 49 mg/100 ml. Despite regular treatment with vitamin B_{12} the Hb fell to 10.5 g/100 ml and erythrocyte count to 2.9 mill./mm³. Roentgen examination of the skeleton showed rarefaction, compression of the 8th thoracic and 1st lumbar vertebrae and osteolytic changes of myeloma type in the skull. Serum protein electrophoresis: Total protein 6.3, albumin 3.9, α_1-globulin 0.49, α_2-globulin 0.71, β_1-globulin 0.36, β_2-globulin 0.25, γ-globulin 0.58 g/100 ml. Since the patient had both hypogammaglobulinaemia and

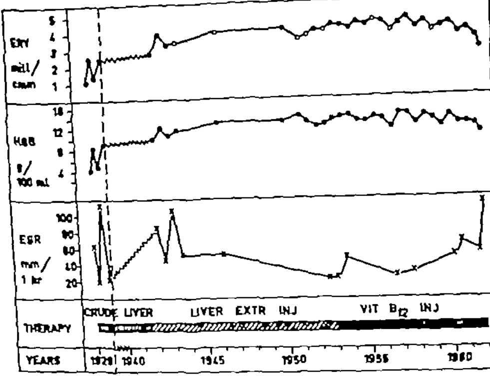


Fig. 1. Case 3. Course of red cell count, haemoglobin concentration and ESR.

proteinuria, the protein pattern of the urine was studied, and it revealed pathological globulin fraction. The sternal bone marrow showed definite increase in the number of plasma cells. Schilling test without intrinsic factor gave an excretion of 2 in 24 hours and with intrinsic factor 5 %. The examination was repeated without and with double dose of intrinsic factor on urine collected for 3 days and excretion values of 2 % + 1 % + + 0 and 9 + 1 % + 3 % respectively were noted. The condition of the patient became worse and he died in January 1961. Post mortem examination showed myeloma. The results of the absorption tests showed that he had intrinsic factor deficiency and that he was partially refractory to intrinsic factor. T our knowledge he had not been treated with intrinsic factor.

Case 4. A woman, born in 1875, who was admitted on Sept. 2, 1959 because of thrombosis of the lower leg. She had also had mild symptoms of cardiac failure but had otherwise always felt well. Physical examination revealed smooth tongue and weak Achilles tendon reflexes, but otherwise nothing of interest. Hb 11.5 g/100 ml, erythrocytes 3.5 mill./mm³ leucocytes 4,100/mm³. Platelets 204,000/mm³. ESR 62 mm/1 hour. Serum protein electrophoresis: Total protein 8.7 albumin 3.96, α_1-globulin 0.38, α_2-globulin 0.64 β_1-globulin 0.43 β_2-globulin 0.26, γ-globulin including a pathological component 3.01 g/100 ml. The sternal bone marrow contained abundant plasma cells. Roentgen examination revealed rarefaction but no definite signs of destruction. Serum vitamin B_{12} 34 $\mu\mu$g/ml. Schilling test showed an excretion of 7 % in 24 hours without intrinsic factor, with intrinsic factor 22 %. Test meal: histamine refractory achlorhydria. Administration of vitamin B_{12} in association with the Schilling test had no effect on the blood values.

The last clinical examination in 1962: no bone pains, no skeletal destruction. The γ-

globulin had increased continuously The diagnosis of myeloma seems very probable but cannot be regarded as definitely proved The vitamin B_{12} absorption tests showed the presence of intrinsic factor deficiency

Case 5. A woman, born in 1887 who had been treated at the hospital in 1929 because of pernicious anaemia after having been troubled for 2 years by fatigue, burning of the tongue and paraesthesiae of the legs. At that time she had a severe hyperchromic anaemia with an Hb of 4.2 g/100 ml and erythrocyte count of 1 mill./mm³ Test meal achlorhydria. Neurological examination revealed probably impaired position sense in the legs. Treatment with raw liver produced considerable improvement In 1941 this was replaced by injection of liver extract and in 1952 by parenteral vitamin B_{12}. Since the beginning of the nineteen forties her resistance against infections had decreased and she had had pneumonia on various occasions. She was admitted on 7 occasions because of pneumonia, and bronchiectasis had also been suspected During these acute infections the ESR had risen to high levels and never returned to normal. The lowest value recorded was 24 mm/1 hour during a symptom free interval in 1953 During pneumonia in the spring of 1960 the ESR noted was 124 mm/1 hour which afterwards fell, but not below 77 mm/1 hour It was therefore decided to study the protein electrophoretic pattern of the serum, which was found to contain an abnormal γ-component of myeloma type. The patient was admitted for investigation in May 1960 Physical examination revealed atrophic papillae of the tongue, and decreased sense of vibration in the legs. Hb 9 9 g/100 ml, erythrocytes 3 3 mill /mm³ leucocytes 5 900/mm³ ESR 97 mm/1 hour Platelets 412,000/mm³ reticulocytes 14 000/mm³ Serum protein electrophoresis total protein 8 4 albumin 2 95 α_1-globulin 0 41 α_2-globulin 0 61 β_1-globulin 0 41 β -globulin 0 31 γ-globulin 0.50 abnormal γ-globulin fraction 3.23 g/100 ml The sternal bone marrow contained a markedly increased number of plasma cells. Roentgen examination of the skeleton showed no signs of myeloma. Test meal histamine refractory achlorhydria. The Schilling test without intrinsic factor 0 8 % excretion per 24 hours with intrinsic factor 3 % and after 7 days oral treatment with tetracycline 2 % before the patient was afterwards treated regularly with vitamin B_{12} parenterally In Aug. 1961 she had another attack of pneumonia. In Sept. 1961 she was again admitted. Hb 9.2 g/100 ml, erythrocytes 2 6 mill./mm³ ESR 11. mm/1 hour Serum protein electrophoresis: Total protein 10 1 albumin 4.08, α_1-globulin 0.26, α_2-globulin 0.54 β_1-globulin 0.34, β_2-globulin 0.28, γ-globulin 0.25, abnormal γ-fraction 4 34 g/100 ml. Bone marrow from a spinal process showed 23.5 % myeloma cells, but roentgenography still showed no changes of myeloma. The Schilling test with intrinsic factor still showed a low excretion — 1 for 24 hours. The serum creatinine was 1.0 mg/100 ml. Histidine tolerance test slightly increased excretion of formimino-glutamic acid. Oral xylose tolerance test decreased absorption with an excretion of 2.5 g (normal value 4—9 g)

The patient developed acute pneumonia in Dec. 1961 and died in spite of treatment with antibiotics. The post mortem examination showed myeloma. The vitamin B_{12} absorption tests and the defective xylose resorption tests suggested that the impaired absorption of B_{12} which had produced clinical symptoms for more than 30 years might have been due to some form of malabsorption syndrome. The B_{12} resorption was, however not tested with a larger dose of intrinsic factor or human gastric juice. Another interesting point in this case history was that the markedly decreased resistance to infections had existed some 20 years before the myeloma had been diagnosed and that ESR had apparently never been normal during this long period.

As mentioned, we have also seen 2 cases of benign monoclonal hyperglobulinaemia with a clinical picture of pernicious anaemia.

Case 6 A chief steward born in 1900, who in 1927 had had amoebic dysentery and afterwards occasional periods of diarrhoea. In 1943 he had been treated in hospital because of achlorhydria. On Feb. 13, 1952 he was admitted because of suspected liver disease Hb 16.2 g/100 ml, erythrocytes 4.5 mill./mm³ leucocytes 7,900/mm³ ESR 10 mm/1 hour Bilirubinaemia 2.55 mg/100 ml negative direct reaction. Thymol ext. 0 42. Total serum protein 7 1 globulin 3.0 g/100 ml. Bilirubin/urine 0 Alk. phos. 5.6 U The case was diag-

preted as representing hepatitis or incipient liver cirrhosis. The patient was re-admitted on Dec. 11 1953 after having been troubled by periods of diarrhoea for 6 months and considerable loss of weight. Physical examination on admission showed reduced amount of body fat and marginal atrophy of the tongue. Hb 8.6 g/100 ml, erythrocytes 1.7 mill./mm³ leucocytes 2,900/mm³ ESR 47 mm/1 hour Thymol ext. 0.25. Fe/s 210 µg/100 ml. Total serum protein 6.5, globulin 2.2 g/100 ml. Platelets 255 000/mm³ Reticulocytes 0.8 %. Sternal puncture megaloblastic bone marrow Test meal histamine refractory achlorhydria. The B_{12}-concentration in the serum was 40 µµg/ml. Oral treatment with vitamin B_{12} produced an increase in the number of reticulocytes to 4.8 % and the serum iron dropped to 101 µg/100 ml. On subsequent treatment with parenteral B_{12} the number of reticulocytes again rose, this time from 1.0 % to 7.4 %. Therapy was continued at the outpatient department and the blood picture became normal. On Feb. 11 the Hb was 12.5 g/100 ml and erythrocytes 4.0 mill./mm³ He was re-admitted on Nov 30, 1954 Hb 13.6 g/100 ml, erythrocytes 4.4 mill./mm³ ESR 18—26 mm/1hour Thymol ext. 0.16. Serum protein electrophoresis total protein 7.2, albumin 3.92, α_1-globulin 0.41 α_2-globulin 0.57 β-globulin 1.40, γ-globulin 0.94 g/100 ml with an abnormal component in the β-globulin fraction. Bromsulphalein retention 11.5 %. In 1955 roentgen examination showed possible rarefaction but no osteolytic changes. During the following years the patient received vitamin B_{12} regularly and the blood values were normal. The ESR was slightly elevated, 31—42 mm/1 hour. At that time he consumed rather large amounts of alcohol, but in June 1958 the bromsulphalein retention was normal, 8 %. In Jan. 1959 serum protein electrophoresis showed the following values total protein 8.3, albumin 4.14 α_1-globulin 0.37 α_2-globulin 0.60 β_1-globulin 0.60 β_2-globulin 1.60, γ-globulin 1.18. The immunological type of the abnormal globulin was found to be β_{2A}. During his last spell in hospital in June 1959 the blood values were still normal, the Hb was 13.9 g/100 ml, and the erythrocytes 4.5 mill./mm³ The ESR had risen still further to 64 mm/1 hour. On June 11, 1960 the patient died at home. There was no examination post mortem.

In this case the serum vitamin B_{12}-value and the response to treatment with vitamin B_{12} showed that the megaloblastic anaemia was due to vitamin B_{12} deficiency. The histamine refractory achlorhydria might argue for an intrinsic factor deficiency as the cause. Even if the patient had a rather high alcohol consumption it is not probable that his diet was so defective that he would acquire dietary B_{12} deficiency. However the possibility of a malabsorption syndrome cannot be excluded because the vitamin B_{12} absorption test was not performed. In addition, the patient had a pathological globulin fraction for 5 years, but neither sternal puncture nor roentgen examination of the skeleton produced any evidence of myeloma.

Case 7. A woman, born in 1882, who, after having had symptoms of cardiac failure for some years was admitted on Oct. 19 1959 because of 6 months tendency to anaemia and an elevated ESR. *On admission* Moderate signs of cardiac failure. Hb 12.1 g/100 ml, erythrocytes 3.3 mill./mm³ leucocytes 8,300 mm³ She had no proteinuria. N P N 29 mg/100 ml. After dehydration clinical improvement was noted. The ESR rose from 26 mm/1 hour on admission to 56 mm/1 hour. The Schilling test without and with intrinsic factor showed an excretion of 7 % and 15 % in 24 hours. Test meal histamine refractory achlorhydria. Serum protein electrophoresis Total protein 8.4, albumin 4.36, α_1-globulin 0.47 α_2-globulin 0.69 β_1-globulin 0.66, β_2-globulin 0.89 γ-globulin 1.34 g/100 ml, with an abnormal component in the β_2-region. Roentgen examination of the skeleton showed no signs of myeloma. The sternal bone marrow was of normal appearance. Test meal histamine refractory achlorhydria. No increase in the number of reticulocytes was noted after the first Schilling test. Later however the blood picture improved, and on Dec. 3 the Hb was 13.0 g/100 ml and the erythrocytes 4.0 mill./mm³

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This patient had an abnormal globulin component in the β-globulin region, but neither the bone marrow nor the skeleton showed any signs of myeloma. The difference in the excretion in the Schilling test with and without intrinsic factor cannot be explained by fluctuations in the severity of her cardiac failure, because she was better during the

globulin had increased continuously The diagnosis of myeloma seems very probable but cannot be regarded as definitely proved. The vitamin B_{12} absorption tests showed the presence of intrinsic factor deficiency

Case 5 A woman born in 1887 who had been treated at the hospital in 1929 because of pernicious anaemia after having been troubled for 2 years by fatigue, burning of the tongue and paraesthesiae of the legs. At that time she had a severe hyperchromic anaemia with an Hb of 4.2 g/100 ml and erythrocyte count of 1 mill./mm³ Test meal achlorhydria. Neurological examination revealed probably impaired position sense in the legs. Treatment with raw liver produced considerable improvement. In 1941 this was replaced by injection of liver extract and in 1952 by parenteral vitamin B_{12}. Since the beginning of the nineteen forties her resistance against infections had decreased and she had had pneumonia on various occasions. She was admitted on 7 occasions because of pneumonia, and bronchiectasis had also been suspected During these acute infections the ESR had risen to high levels and never returned to normal. The lowest value recorded was 24 mm/1 hour during a symptom free interval in 1953 During pneumonia in the spring of 1960 the ESR noted was 124 mm/1 hour which afterwards fell, but not below 77 mm/1 hour It was therefore decided to study the protein electrophoretic pattern of the serum, which was found to contain an abnormal γ-component of myeloma type. The patient was admitted for investigation in May 1960 Physical examination revealed atrophic papillae of the tongue, and decreased sense of vibration in the legs. Hb 9.9 g/100 ml, erythrocytes 3.3 mill /mm³ leucocytes 5,900/mm³ ESR 97 mm/1 hour Platelets 412,000/mm³ reticulocytes 14 000/mm³ Serum protein electrophoresis. total protein 8 4 albumin 2 95 α_1-globulin 0 41 α_2-globulin 0 61 β_1-globulin 0.41 β-globulin 0 31 γ-globulin 0 50, abnormal γ-globulin fraction 3.23 g/100 ml. The sternal bone marrow contained a markedly increased number of plasma cells. Roentgen examination of the skeleton showed no signs of myeloma. Test meal histamine refractory achlorhydria. The Schilling test without intrinsic factor 0 8 % excretion per 24 hours with intrinsic factor 3 % and after 7 days oral treatment with tetracycline 2 %. As before the patient was afterwards treated regularly with vitamin B_{12} parenterally In Aug 1961 she had another attack of pneumonia. In Sept. 1961 she was again admitted. Hb 9.- g/100 ml, erythrocytes 2.6 mill./mm³ ESR 112 mm/1 hour Serum protein electrophoresis Total protein 10.1 albumin 4.08, α_1-globulin 0.26 α_2-globulin 0.54 β_1-globulin 0.34, β_2-globulin 0.28 γ-globulin 0.25, abnormal γ-fraction 4.34 g/100 ml. Bone marrow from a spinal process showed 23.5 % myeloma cells but roentgenography still showed no changes of myeloma. The Schilling test with intrinsic factor still showed a low excretion — 1 % for 24 hours. The serum creatinine was 1.0 mg/100 ml. Histidine tolerance test slightly increased excretion of formimino-glutamic acid. Oral xylose tolerance test decreased absorption with an excretion of 2.5 g (normal value 4—9 g)

The patient developed acute pneumonia in Dec. 1961 and died in spite of treatment with antibiotics. The post mortem examination showed myeloma. The vitamin B_{12} absorption tests and the defective xylose resorption suggested that the impaired absorption of B_{12} which had produced clinical symptoms for more than 30 years might have been due to some form of malabsorption syndrome. The B_{12} resorption was, however not tested with a larger dose of intrinsic factor or human gastric juice. Another interesting point in this case history was that the markedly decreased resistance to infections had existed some 20 years before the myeloma had been diagnosed and that ESR had apparently never been normal during this long period.

As mentioned, we have also seen 2 cases of benign monoclonal hyperglobulinaemia with a clinical picture of pernicious anaemia.

Case 6 A chief steward, born in 1900, who in 1927 had had amoebic dysentery and afterwards occasional periods of diarrhoea. In 1943 he had been treated in hospital because of achlorhydria. On Feb. 13, 1952 he was admitted because of suspected liver disease Hb 16.2 g/100 ml, erythrocytes 4.5 mill./mm³ leucocytes 7,900/mm³ ESR 10 mm/1 hour Bilirubins 2.55 mg/100 ml negative direct reaction. Thymol ext. 0 42. Total serum protein 7 1 globulin 3 0 g/100 ml. Bilirubin/urine 0. Alk. phos. 5.6 U The case was diag-

nosed as regressing hepatitis or incipient liver cirrhosis. The patient was re-admitted on Dec. 11, 1953 after having been troubled by periods of diarrhoea for 6 months and considerable loss of weight. Physical examination on admission showed reduced amount of body fat and marginal atrophy of the tongue. Hb 8.6 g/100 ml, erythrocytes 1 7 mill./mm³ leucocytes 2,900/mm³ ESR 47 mm/1 hour Thymol ext. 0.25. F /s 210 μg/100 ml. Total serum protein 6.5, globulin 2.2 g/100 ml. Platelets 255,000 mm³ Reticulocytes 0.8 %. Sternal puncture megaloblastic bone marrow Test meal histamine refractory achlorhydria. The B_{12}-concentration in the serum was 40 $\mu\mu$g/ml. Oral treatment with vitamin B_{12} produced an increase in the number of reticulocytes to 4.8 % and the serum iron dropped to 101 μg/100 ml. On subsequent treatment with parenteral B_{12} the number of reticulocytes again rose this time from 1.0 % to 7.4 %. Therapy was continued at the outpatient department and the blood picture became normal. On Feb. 11 the Hb was 12.5 g/100 ml and erythrocytes 4.0 mill./mm³ He was re-admitted on Nov 30, 1954 Hb 13.6 g/100 ml, erythrocytes 4 4 mill./mm³ ESR 18—26 mm/1hour Thymol ext. 0.16. Serum protein electrophoresis total protein 7.2, albumin 3.92, α_1-globulin 0.41 α_2-globulin 0.57 β-globulin 1 40, γ-globulin 0 94 g/100 ml with an abnormal component in the β-globulin fraction. Bromsulphalein retention 11.5 %. I 1955 roentgen examination showed possible rarefaction but no osteolytic changes. During the following years the patient received vitamin B_{12} regularly and the blood values were normal. The ESR was slightly elevated, 31—42 mm/1 hour At that time he consumed rather large amounts of alcohol, but in June 1958 the bromsulphalein retention was normal, 8 %. I Jan. 1959 serum protein electrophoresis showed the following values total protein 8.3, albumin 4 14 α_1-globulin 0.37 α_2-globulin 0.60, β_1-globulin 0.60, β_2-globulin 1.60, γ-globulin 1 16. The immunological type of the abnormal globulin was found to be β_{2A}. During his last spell in hospital in June 1959 the blood values were still normal, the Hb was 13.9 g/100 ml, and the erythrocytes 4.5 mill./mm³ The ESR had risen still further to 64 mm/1 hour On June 11 1960 the patient died at home. There was no examination *post mortem*.

In this case the serum vitamin B_{12}-value and the response to treatment with vitamin B_{12} showed that the megaloblastic anaemia was due to vitamin B_{12}-deficiency The histamine refractory achlorhydria might argue for an intrinsic factor deficiency as the cause. Even if the patient had a rather high alcohol consumption it is not probable that his diet was so defective that he would acquire a dietary B_{12} deficiency However the possibility of a malabsorption syndrome cannot be excluded because the vitamin B_{12} absorption test was not performed. In addition, the patient had a pathological globulin fraction for 5 years, but neither sternal puncture nor roentgen examination of the skeleton produced any evidence of myeloma.

Case 7 A woman, born in 1882, who, after having had symptoms of cardiac failure for some years was admitted on Oct. 19 1959 because of 6 months tendency to anaemia and an elevated ESR. O admission, Moderate signs of cardiac failure. Hb 12.1 g/100 ml, erythrocytes 3.5 mill./mm³ leucocytes 8,500/mm³ She had no proteinuria. N P N 29 mg/100 ml. After dehydration clinical improvement was noted. The ESR rose from 26 mm/1 hour on admission to 56 mm/1 hour The Schilling test without and with intrinsic factor showed an excretion of 7 and 13 in 24 hours. Test meal histamine refractory achlorhydria. Serum protein electrophoresis Total protein 8.4 albumin 4.36, α_1-globulin 0.47 α_2-globulin 0.69 β-globulin 0.66, β globulin 0.89 γ-globulin 1.34 g/100 ml, with an abnormal component in the β_2-region. Roentgen examination of the skeleton showed no signs of myeloma. The sternal bone marrow was of normal appearance Test meal histamine refractory achlorhydria. No increase in the number of reticulocytes was noted after the first Schilling test. Later however the blood picture improved, and on Dec. 3 the Hb was 13.0 g/100 ml and the erythrocytes 4.0 mill./mm³

This patient had an abnormal globulin component in the β-globulin region, but neither the bone marrow nor the skeleton showed any signs of myeloma. The difference in the excretion in the Schilling test with and without intrinsic factor cannot be explained by fluctuations in the severity of her cardiac failure, because she was better during the

test without intrinsic factor than when the test was performed with intrinsic factor. She thus appeared to have intrinsic factor deficiency. It is noteworthy that she did not have any reticulocyte crises in association with the first Schilling test, but the blood picture improved successively which can probably be ascribed to the injections of vitamin B_{12} in association with the Schilling tests.

As mentioned above, 2 cases that had been treated elsewhere and had shown a picture highly suggestive of pernicious anaemia were also studied. One of these cases had a serum protein disorder mainly of the type of benign monoclonal hyperglobulinaemia (No. 8) and the other one a clear-cut myeloma (No. 9).

Case 8. A housewife, born in 1892, who had been cared for at the Medical department of the County Hospital in Kristinehamn. In the spring of 1959 the patient was troubled by tenderness and swelling of the lips and soreness of the mouth, and since the summer of 1959 she had gradually been losing weight. She consulted her family doctor who diagnosed anaemia. She was treated with Bendogen tablets (vitamin B_{12} and hog pyloric mucosa) and the blood values became normal. In Jan. 1960 she stopped taking Bendogen. She was admitted on Feb. 25, 1960. On admission the tongue was found to be red and smooth and the oral mucosa was reddened with white spots. Hb 10.5 g/100 ml, erythrocytes 3.1 mill./mm³, leucocytes 5 600/mm³. Platelets 295,000/mm³. Reticulocytes 1.1 %. Proteinuria 0.3—0.7 pro mille. Test meal: histamine refractory achlorhydria. Fe/s 108 μg/100 ml. Schilling test: 2.7 % excretion on first day and 1.4 % on second day. Sternal puncture normal. ESR 47 mm/1 hour. Serum protein electrophoresis: Total protein 9.5, albumin 4.86, α_1-globulin 0.46, α_2-globulin 0.71, β_1-globulin 0.57, β_2-globulin 0.36, γ-globulin 0.84, abnormal γ-globulin fraction 1.71 g/100 ml. Roentgen examination of the spine and the pelvis showed moderate generalized rarefaction. The skull was not examined. From Feb. 29 to March 10 the patient was treated with 1 000 μg vitamin B_{12} per day parenterally and the reticulocyte count increased to 4 %. During follow-up at the out-patient department she was treated with vitamin B_{12} injections every month. On April, 29 the Hb was 11.1 g/100 ml and the erythrocyte count 3.2 mill./mm³. On July 26 the corresponding values were 11.1 g/100 ml and 3.6 mill./mm³ respectively. During treatment with vitamin B_{12} the tongue and the mucosa became better.

The primary good response to vitamin B_{12} and the Schilling test showed that the patient had had a B_{12}-deficiency on the basis of an absorption disorder. The histamine refractory achlorhydria might argue for pernicious anaemia, but since the Schilling test with intrinsic factor was not performed, other disturbances of absorption cannot be excluded. The abnormal protein fraction in the serum was not accompanied by any signs of myeloma in the roentgenogram of the skeleton or in the sternal bone marrow. It is, however, noteworthy that the patient's brother died from a γ-myeloma.

Case 9. The patient was a woman, born in 1889, who had been cared for at the Medical department of the County Hospital in Kristinehamn. Achlorhydria had been diagnosed in 1951, and between 1953 and 1958 she had been admitted to hospital three times for gynaecological and gastrointestinal disorders. On those occasions the ESR and Hb were normal. In the autumn of 1959 she complained of increasing fatigue. In Dec. 1959 fever supervened and in Jan. 1960 she had chest pain. On admission on Jan. 28, 1960, Hb 3.7 g/100 ml, erythrocytes 1.0 mill./mm³, leucocytes 2 400/mm³. Reticulocytes 1.4 %. Fe/s 202 μg/100 ml. ESR 143 mm/1 hour. No proteinuria. N.P.N. 30 mg/100 ml. The sternal bone marrow was found to contain numerous plasma cells and megaloblasts. Serum B_{12} 24 $\mu\mu$g/ml. Serum protein electrophoresis: Total protein 7.3, albumin 3.32, α_1-globulin 0.47, α_2-globulin 0.57, β_1-globulin 0.41, β_2-globulin 0.31, γ-globulin 0.40, abnormal γ-fraction 1.82 g/100 ml. Test meal: histamine refractory achlorhydria. Roentgen examination of the thoracic spine showed generalized rarefaction. Treatment with 200 μg vitamin B_{12} per day for 3 weeks produced considerable improvement. The serum iron fell to 51 μg/100 ml. The reticulocyte count rose to 15.8 % and when the patient left hospital on March 23 the Hb was 1?.2 g/100 ml and erythrocytes 3.7 mill./mm³. She was afterwards treated with 200 μg B_{12} every third week and was readmitted in July 1960 for observation.

Examination revealed Hb 13.3 g/100 ml, erythrocytes 4.2 mill./mm³ ESR 30 mm/1 hour. Roentgenographical evidence of myeloma was found in the skull, pelvis and upper arms as well as destruction with compression of the 6th and 7th thoracic, and 1st and 2nd lumbar vertebrae. The Schilling test showed an excretion of 7.4 % on the first day and of 2.3 % on the second.

This patient thus had myeloma and megaloblastic anaemia on the basis of a vitamin B_{12} deficiency. The Schilling test showed subnormal excretion, but since the examination with intrinsic factor had not been performed it is not possible to say with certainty whether she had intrinsic factor deficiency.

Discussion

Of the 69 consecutive patients with myeloma in Malmö, 3 were thus found to have intrinsic factor-deficiency, i. e. pernicious anaemia and 1 probably had pernicious anaemia. This would mean 4.3 % and 5.8 %, respectively. During the 10 year period studied, i. e. 1951—1960 123 cases of pernicious anaemia were discovered at the Medical Department and 15 at the Department for Epidemic Diseases, making a total of 138. This figure probably includes all cases of untreated pernicious anaemia admitted to the hospital during the period under discussion. The mean was 6.2 per 100 000 inhabitants per year. This frequency is somewhat lower than that found by Nordenson et al. (17) 1938 for the district of Malmöhus in an investigation of the geographical distribution of pernicious anaemia in Sweden. For 1931—1935 they gave a mean of 7.36 per 100 000 inhabitants per year. But as mentioned, their investigation covered not only the town of Malmö but the entire county. We know that the diagnosis was not so accurate at this time, when many patients with e. g. haemolytic anaemia were called pernicious anaemia. The slight and possible difference might also be explained to some extent by the fact that the treatment of pernicious anaemia was considerably simplified during the nineteen-fifties after vitamin B_{12} had become available. It is possible that mild cases had from the beginning been treated by general practitioners and never been referred to the hospital. Although the frequency found by us, i. e. 0.062 % might have been somewhat too low it was accepted as a measure of the incidence of pernicious anaemia in Malmö. On comparison of the frequency of pernicious anaemia in our myeloma series with that for the population of the town, the difference will be statistically significant ($p < 0.001$). Our patients with myeloma were, however fairly old and pernicious anaemia is more common in higher age classes. Thus the coincidence might have been due to chance, but an aetiological relationship between the two diseases cannot be excluded.

There is no accepted reason to suppose that pernicious anaemia may give rise to myeloma, but one might imagine that myeloma could contribute to the development of a vitamin B_{12} deficiency. It was previously suggested that in primary hypogammaglobulinaemia the decreased antibacterial resistance may result in changes of the intestinal flora, which may precipitate pernicious anaemia in constitutionally predisposed individuals (12). It is well known that the bacterial flora in the intestines is changed in patients with pernicious anaemia (3) and that the intestinal flora may influence the absorption of vitamin B_{12} (5, 6, 19). Owing to their low content of normal gammaglobulins patients with myeloma

are immunologically comparable with patients with hypogammaglobulinaemia (18 24) It is therefore possible that myeloma may, through some mechanism resembling that suggested for a possible connection between hypogammaglobulinaemia and pernicious anaemia (12) produce vitamin B_{12}-deficiency in individuals with inherited or acquired defective vitamin B_{12}-absorption The time relationship between the two diseases in case No 5 does not necessarily argue against this line of thought. In that case the anaemia appeared 30 years before the myeloma was diagnosed. The antibacterial resistance had, however been obviously decreased for about 20 years. One might therefore wonder how long the patient had had her myeloma before it had been diagnosed (serum protein electrophoresis was not done until the myeloma was diagnosed) and whether myeloma might not begin as a benign condition It might be mentioned, that on one occasion one of our patients with hypogammaglobulinaemia had an abnormal narrow gamma globulin band in the electrophoretic pattern (12) and that Lindholm (14) has described a case of hypogammaglobulinaemia with myeloma in the family

Another possibility which may be worth considering is that the production of abnormal globulin may be able to play a rôle in the development of vitamin B_{12} deficiency It has been shown that the serum vitamin B_{1} -concentration might be decreased in the presence of myeloma despite normal absorption of B_{12}, possibly as a consequence of the production of pathological proteins resulting in an increased consumption of vitamin B_{1} (11) It was found that in patients with large amounts of pathological globulin the serum vitamin B_{12} tended to be low (11) An increased B_{12}-consumption might possibly precipitate a vitamin B_{12}-deficiency in persons who have previously had a disorder of the vitamin B_{12} absorption but not yet developed clinical symptoms.

Finally the possibility of both the above mentioned factors playing a rôle in causing vitamin B_{12}-deficiency in myeloma may also be considered

Summary

A description is given of 6 cases of myeloma and 3 cases of benign monoclonal hyperglobulinaemia, all with a clinical picture of pernicious anaemia. Five of the cases of myeloma were part of a series of 69 cases collected from Malmö General Hospital during a 10-year period.

In 2 cases the vitamin B_{12} absorption was not studied, but in one of them the serum B_{12} level was known to be low and in the other the clinical course strongly suggested vitamin B_{12} deficiency In the other 7 cases the Schilling test gave low values and in 4 of the 5 in which the test was repeated with intrinsic factor the absorption became normal.

The possibility of an aetiological relationship between these protein disorders and vitamin B_{12} deficiency is discussed.

Acknowledgements

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Splitting of the Rheumatoid Factor without Loss of its Haemagglutinating Activity

By

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A preliminary report on the possibility of modifying the macroglobulin which contains the rheumatoid factor without loss of its ability to agglutinate sensitized erythrocytes was published in this journal 1961 (1). A more detailed description of these experiments is given below.

That the RF macroglobulin can be broken down into smaller molecules is self-evident. But, in being split, its haemagglutinating activity usually disappears. For several years Svartz has been trying different methods of splitting the RF macroglobulin without this loss of its haemagglutinating activity. This proved possible by using ammonium sulphate in 1.23 molecular concentration.

The procedure is as follows. Serum from rheumatoid arthritis patients in whom the Waaler Rose test was positive, is fractionated with 1.23 mol. $(H_4N)_2SO_4$. The supernatant fluid is removed. The haemagglutination test is done on the precipitate redissolved in phosphate buffer (pH 7.4). The haemagglutination titre was usually found to be the same as or slightly below that of the original serum.

Part of the same fluid as was tested for haemagglutination was subjected to ultracentrifugation. Fig. 1 shows the ultracentrifugation of original serum. In this case a macroglobulin was found with a sedimentation coefficient of 18.7 S. Fig. 2 demonstrates the result of ultracentrifugation of redissolved ammonium sulphate precipitate from the same serum. In this precipitate there is still a peak with about 18.7 S, but it is considerably lower than in the original serum. In addition, a new peak with 14 S has been formed. The haemagglutination titre was unchanged.

Of special importance are the next experiments. Fig. 3 shows original serum with a macroglobulin with coefficient 18.6 S. After $(H_4N)_2SO_4$ fractionation of this serum the macroglobulin peak with 18.6 S is found to have completely disappeared, as seen from fig. 4. The haemagglutinating activity is nevertheless retained. The new peak has a sedimentation coefficient of 15.9 S. The quantity of 4—7 S material has increased. Figs. 5 and 6 show similar results in an-

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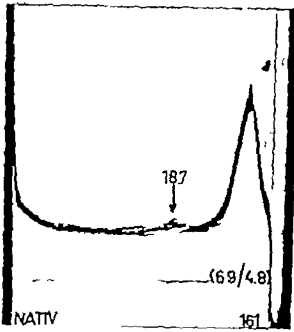


Fig 1 Ultracentrifugation of rheumatoid serum. The rheumatoid factor is bound to the 18.7 S peak.

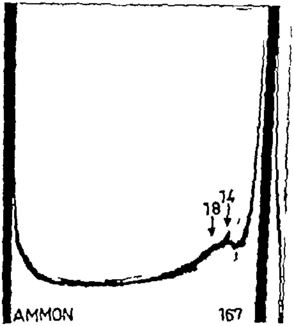


Fig 2. Ultracentrifugation of ammonium sulphate precipitate from same serum as in fig 1

other case. Fig 7 demonstrates an experiment in which two peaks with lower S-value have appeared instead of 19.3 S As far as is known it has not previously been demonstrated that a physico-chemical

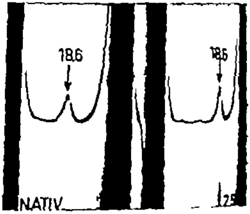


Fig 3 Haemagglutinating activity (rheumatoid factor) is associated with the 18.6 S peak (untreated serum)

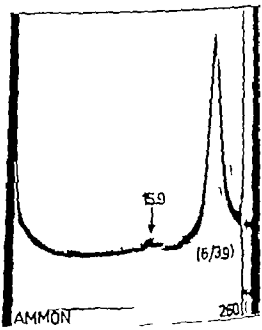


Fig 4 Ammonium sulphate precipitate from same serum as in fig 3 No 18—19 S peak can be seen. A peak showing lower S (15.9) now contains the haemagglutinating activity

change of the macroglobulin containing the RF can take place without affecting its haemagglutinating activity

It was naturally desirable to try to find out whether the same phenomenon could be demonstrated in prepurified RF macroglobulin The following procedure was employed Original serum, diluted 1:4

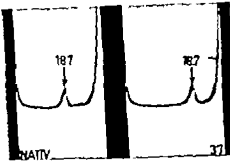


Fig. 5. Haemagglutinating activity in 18.7 S peak (untreated serum).

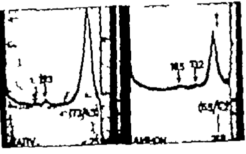


Fig. 7. Original serum to the left, ammonium sulphate precipitate to the right.

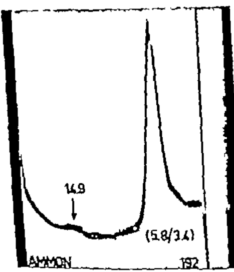


Fig. 6. Ammonium sulphate precipitate from same serum as in fig. 5. Haemagglutinating activity in 14.9 S peak.

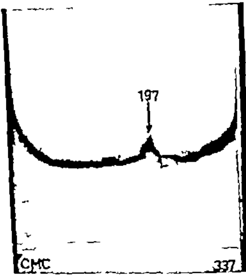


Fig. 8. Ultracentrifugation of purified RF-macroglobulin. Haemagglutinating activity in 19.7 S peak.

times with distilled water was fractionated in the cold (+ 4 C for 48 hours). The supernatant was removed. The precipitate was dissolved in phosphate buffer (pH 7.4) and chromatographed in a column containing the cation exchanger carboxy-methyl-cellulose. The fraction showing the highest haemagglutination titre was ultracentrifuged. Fig 8 shows a case in which the sedimentation coefficient of the purified macroglobulin was unusually high, 19.7 S. Part of the same fraction was precipitated with ammonium sulphate. The redissolved precipitate showed roughly as strongly positive a sheep cell test as the isolated macroglobulin in fig. 8. On ultracentrifugation the 19.7 S fraction was no longer found, but, instead, a peak with 16.9 S, i. e. nearly three units lower than before the ammonium sulphate fractionation (fig 9). Thus the same phenomenon was re-

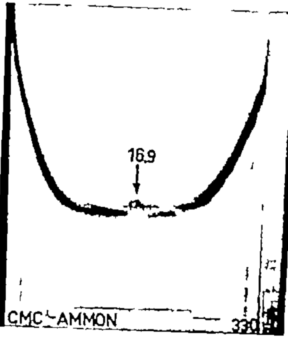


Fig 9 Ammonium sulphate precipitate from the purified factor in fig 8. Haemagglutinating activity in 16.9 S peak.

vealed with the isolated factor as with the original serum. The carbohydrate content of the ammonium sulphate precipitate was 7 per cent.

Summary

The experiments recounted above show that the macroglobulin which contains the rheumatoid factor can be split without loss of its haemagglutinating activity

The method employed in these experiments was fractionation with 1.23 mol. ammonium sulphate. Two types of processes were observed. In certain experiments there was a duplication of the macroglobulin peak shown by the original serum. Thus, there remains a residue of a fraction with the same sedimentation coefficient as in the original serum, but, in addition, a new fraction appears with 3—5 units lower coefficient. In other experiments the original macroglobulin peak disappeared entirely and a fraction with lower coefficient emerged. The haemagglutinating activity remained.

The same phenomenon was demonstrated in ammonium sulphate fractionation of isolated RF macroglobulin.

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The Blood-gas Exchange and the pH in Chronic Pulmonary Heart Disease

By

S. Daum, F Herles and M. Kopecky

The type and degree of respiratory insufficiency is characterized by the values for the blood-gas exchange and for the acid-base equilibrium. Baldwin Cournand and Richards (1) as well as Rossier Bühlman and Wiesinger (11) took these values as the basis for their classification of the respiratory insufficiency to which we also adhere. The blood-gas exchange serves also as the basis for the calculation of some respiratory values (4) The values for blood-gas exchange in chronic pulmonary heart disease (in tables and figures cor pulmonale chronicum) associated with pulmonary emphysema, are neither uniform nor constant. For the understanding of the sequelae of respiratory insufficiency it is valuable to express the blood-gas exchange in terms of pressure and of the change in blood pH.

These measurements revealed in our experiments a striking relationship of the respiratory function in chronic pulmonary heart disease associated with emphysema to the development of decompensation of cor pulmonale.

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Method and procedure

Patients with chronic pulmonary heart disease associated with obstructive emphysema of the lungs wer divided into three groups. The first group included 16 patients with compensated pulmonary heart disease (CPC) the second 20 patients with decompensated pulmonary heart disease (CPD) and the third included 15 recompensated patients (CPR) The diagnosis of pulmonary heart disease was based on the previous history X-ray and ECG findings and catheterization (6, 14) In patients with compensated pulmonary heart disease (19 examinations on 16 patients) the previous history never included data relative to decompensation (oedema of the lower extremities, hepatomegaly hepatojugular reflux) the X-ray film showed signs of pulmonary hypertension, the heart was not enlarged. During catheterization (in 12 patients) the pulmonary mean pressure at rest (mAP) equalled or exceeded 20 mm Hg The right-ventricular diastolic pressur was below 7 the mean right-auricular pressure (mAD) was below 6 mm Hg In 20 patients with decompensated pulmonary heart disease 34 examinations were carried out. Some of these

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Table I Mean values and sigma observed throughout 1960 up to June 1961 in normal subjects and in individual groups of patients with pulmonary heart disease and with emphysema alone

Diagnosis	No. of pat.	Sa_{O_2} %	Cap_{O_2} vol. %	Pa_{O_2} mm Hg	Ca_{CO_2} vol. %	Pa_{CO_2} mm Hg	pH
Normal	15	96—98	21—22	95	45—55	35—44	7.35—7.[illegible]
Emphysema pulmonum	4	95.2 ± 4.1	20.82 ± 3.6	90.2 ± 13.1	52.75 ± 9.26	49.9 ± 4.0	7.350 ± 0.019
Cor pulmonale compensatum	19	88.50 ± 3.9	21.40 ± 2.5	61.66 ± 12.1	62.1 ± 7.5	50.80 ± 7.7	7.345 ± 0.40
Cor pulmonale decompensatum	34	69.40 ± 11.0	22.1 ± 3.5	43.30 ± 8.8	72.0 ± 8.7	65.6 ± 9.7	7.292 ± 0.029
Cor pulmonale recompensatum	17	89.10 ± 4.10	22.21 ± 4.37	67.73 ± 11.10	66.40 ± 7.40	58.10 ± 8.2	7.366 ± 0.046

patients still presented during these examinations signs of oedema of the lower extremities, a definite liver enlargement, a positive hepatojugular reflux, and an X ray evidence of pulmonary hypertension and in a high proportion of cases an enlarged heart shadow. During catheterisation (19 patients) the mean pressure at rest in the pulmonary artery exceeded 20 mm Hg, and either the diastolic right ventricular pressure was above 7 mm Hg, or the mAD was above 6 mm Hg. In the third group of patients with recompensated pulmonary heart disease (17 examinations in 13 patients) either the previous history included at least one episode of decompensation, or there were signs of decompensation on admission. However during the investigation there were no more signs of an overt decompensation, with the exception of liver enlargement. During catheterization (in 12 patients) the mAP exceeded 20 mm Hg and both the right ventricular diastolic pressure and the mAD were normal. We were compelled to separate the group of patients with recompensated pulmonary heart disease from the group of compensated cases, because of the changes in the blood-gas exchange which did not justify including these patients either in the group of decompensated pulmonary heart disease, or in the group of compensated pulmonary heart disease.

The average age of the patients studied was 49.5 years, and ranged from 26 up to 68 years. The series included 8 women and 41 men.

The oxygen saturation of the arterial blood was estimated in a cuvette of Waters oximeter, the haemoglobin oxygen capacity was calculated from the haemoglobin concentration by means of a factor of 1.34, oxygen tension in the arterial blood (Pa_{O_2}) was estimated with the help of a nomogram according to Severinghaus (12) from the value for oxygen saturation, from the pH and from the rectal temperature of the patient, or by a direct measurement with Bartels' haemoxytensometer manufactured by Eschweiler (2). The content of free carbon dioxide was calculated according to Milch (8) from the values of bound carbon dioxide determined with the van Slyke titration method (13). Pa_{CO_2} was calculated from the Ca_{CO_2} (Mm/l) and from the pH, either according to Milch (8) or by means of the Rahn Fenn nomogram (10).

The blood samples for the analysis of blood-gas and for the determination of the blood pH were obtained by the usual techniques (7). The patients were checked continuously throughout the procedure in an attempt to ascertain whether arterial puncture which sometimes may be painful, is not associated with hyperventilation which would distort the level of the blood-gases (9). The analysis of the blood sample was performed within 5 minutes after the withdrawal of blood.

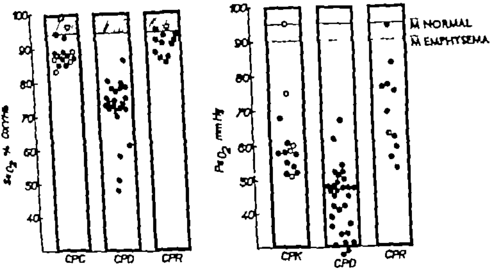


Fig. 1. Values for oxygen saturation (Sa_{O_2}) and tension (Pa_{O_2}) of arterial blood in patients with compensated (CPC), decompensated (CPD) and recompensated (CPR) pulmonary heart disease. ○ Normal values of pH and Pa_{CO_2}. ◐ Hypercapnia with normal values of pH. ● Respiratory acidosis.

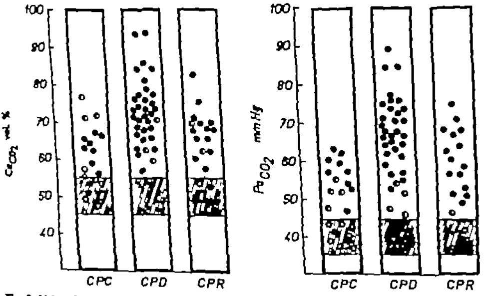


Fig. 2. Values for carbon dioxide content (Ca_{CO_2}) and tension (Pa_{CO_2}) of arterial blood in patients with compensated (CPC) decompensated (CPD), and recompensated (CPR) pulmonary heart disease. ○ Normal pH and blood-gas exchange. ◐ Compensated respiratory acidosis. ● Noncompensated respiratory acidosis.

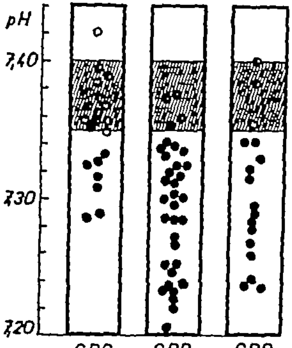


Fig 3. pH in individual patients in compensated, decompensated and recompensated pulmonary heart disease. Symbols as in fig 1

Results

The average values of the blood-gas exchange and pH with standard deviations in individual groups of patients are summarized in table I For the purpose of comparison, values in normal subjects and in patients with emphysema without any evidence of pulmonary heart disease are included in the table.

Oxygen saturation of arterial blood (Sa_{O_2}) and oxygen tension in the arterial blood (Pa_{O}) in patients with compensated with decompensated and with recompensated pulmonary heart disease are shown in fig 1 The white circles indicate cases with normal Pa_{CO_2} and pH values, the semi-circles represent patients with a normal pH (7.35—7 40) but with an elevated Pa_{CO} (44—60 mm Hg) i. e with compensated respiratory acidosis (5) Compared to the other two groups, the patients with decompensated pulmonary heart disease showed a highly significant reduction of saturation. In recompensated pulmonary heart disease the distribution of values for oxygen saturation is similar to that in cases with compensated pulmonary heart disease. There is however a predominance of cases of noncompensated respiratory acidosis. Although in cases with compensated pulmonary heart disease the saturation was about 88 %, the oxygen tension was markedly reduced. Patients with non-compensated respiratory acidosis have a relatively higher tension than the other groups studied.

The carbon dioxide content (Ca_{CO_2}) and the carbon dioxide tension (Pa_{CO_2}) of arterial blood are shown in fig 2. In compensated pulmonary heart disease most of these values are above the upper limit of the norm but some were within the limits of normal. In decompensated pulmonary heart disease all patients had values above the upper limit of the norm, exceeding in a high proportion 60 mm Hg The maximum values of Ca_{CO_2} were 93 volume per cent (2 times) and of the Pa_{CO} 89 mm Hg (in one case) The values in recompensated pulmonary heart disease were widely scattered — but almost all were elevated, and most of the patients had a noncompensated respiratory acidosis. The values for the total volume of Ca_{CO_2} and Pa_{CO_2} are statistically significantly elevated compared to the values in the group with compensated pulmonary heart disease

Fig 3 shows the pH values in patients with compensated decompensated and recompensated pulmonary heart disease In decompensated pulmonary heart disease there is a shift of the pH to the acid side In recompensated pulmonary heart disease there is also a predominance of reduced pH values. In this respect there

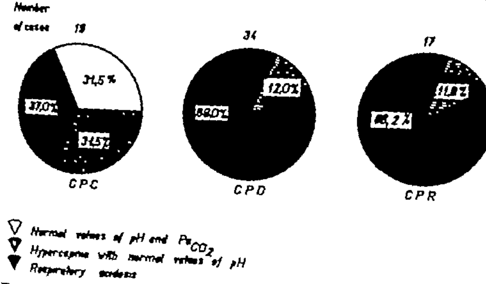


Fig. 4. The frequency of compensated and noncompensated respiratory acidosis, of normal pH and Pa_{CO_2} in patients with pulmonary heart disease in %

is also a statistically significant difference, in contrast to the values observed in compensated pulmonary heart disease.

Discussion

Chronic hypoxia with a deficient oxygen saturation represents one of the conditions leading to the development of pulmonary hypertension. In emphysema of the lungs and in compensated pulmonary heart disease the oxygen saturation is about 90 % and the oxygen tension of arterial blood is distinctly decreased. In both compensated and noncompensated acidosis the oxygen tension is relatively higher (fig. 1). There was no statistically significant abnormality of the carbon dioxide tension and of the pH in patients with simple emphysema of the lungs and in the group with compensated pulmonary heart disease.

In some patients with compensated pulmonary heart disease there is a normal oxygen saturation, as well as Pa_{CO_2} and pH. In our opinion these are patients whose respiratory centre is still very sensitive both to hypoxaemia (chemoreflex drive) and to Pa_{CO_2} (centrogenic drive) (5) so that they are able to maintain a normal oxygen saturation with the help of an elevated alveolar ventilation and thus remove the excess of carbon dioxide by ventilation.

There is however a substantial difference between patients with a simple emphysema, or with compensated pulmonary heart disease, and patients with decompensated pulmonary heart disease. Markedly deficient oxygen saturation with an extremely low oxygen tension and noncompensated respiratory acidosis are characteristic for decompensated pulmonary heart disease. In recompensated pulmonary heart disease the oxygen saturation reverts towards normal, as well as the Pa_{O_2}. Th values of Ca_{CO_2} and of Pa_{CO_2} remain, however elevated and the

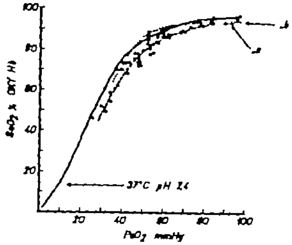


Fig 5. Haemoglobin dissociation curve with points marking the actual oxygen saturation and tension of arterial blood, in individual patients with pulmonary heart disease and normal pH and Pa_{CO} values, or with compensated respiratory acidosis (line b) and in a group of patients with noncompensated respiratory acidosis (line a)

pH reduced often in spite of intensive therapy In most patients with recompensated pulmonary heart disease a chronic respiratory acidosis as described in the classification by American authors is present (1) Its incidence in individual groups is shown in fig 4 It is interesting that all these patients with chronic respiratory acidosis (even with a noncompensated one) are often subjectively in very good health, whilst in patients with acute respiratory insufficiency a noncompensated respiratory acidosis represents besides the perturbation of oxygen saturation the main source of discomfort. These facts indicate that patients with chronic respiratory acidosis, well tolerated adapt themselves not only to hypoxaemia — which after recompensation is restored nearly to normal values — but above all to the chronic respiratory acidosis itself

As is shown in fig 5 acidosis in patients with chronic pulmonary heart disease influences oxygen tension. In the presence of acidosis the Pa_{CO_2} is higher than would be expected according to the oxyhaemoglobin dissociation curve at a normal pH. This is the consequence of Bohr's effect consisting in a slight shift of the curve to the right. In patients with chronic pulmonary heart disease it is thus possible to distinguish two courses of the dissociation curve. One of them is based on the values for oxygen saturation in the presence of a rising oxygen tension in noncompensated respiratory acidosis (fig 5 curve "a) and the second is created by the values for oxygen saturation obtained in respiratory acidosis (semi-circles) or with a normal Pa_{CO_2} as well as pH, and which corresponds to the course of a normal curve (fig 5 curve "b) The shift of the dissociation curve to the right is favourable for the peripheral utilization of oxygen during hypoxaemia.

Patients with chronic pulmonary heart disease associated with emphysema of the lungs show marked differences of blood-gas exchange and pH according to the stage of the disease. Oxygen saturation is only slightly reduced in patients in the compensated stage, whilst oxygen tension is already markedly below normal. The carbon dioxide tension and content are slightly elevated generally not exceeding 60 mm Hg and in some patients normal values may be found. The pH is normal, or in a minority of cases slightly reduced. Patients in the decompensated stage show a markedly reduced oxygen saturation and tension. Both the carbon dioxide tension and content are elevated and in the majority of cases exceed 60 mm Hg The pH is usually reduced below 7.30 as is always found in noncompensated respiratory acidosis. In recompensated patients oxygen saturation is either restored towards normal or remains only slightly

reduced, in contrast to oxygen tension which is markedly reduced. The carbon dioxide tension and content remain elevated, and patients with a pH below 7.35 have respiratory acidosis, usually noncompensated. In these patients an adaptation to the chronic respiratory acidosis is presumed.

Summary

Investigations on the blood-gas exchange and pH were carried out in compensated (16 patients) decompensated (20 patients) and recompensated (15 patients) chronic pulmonary heart disease associated with emphysema of the lungs.

In patients with compensated chronic pulmonary heart disease oxygen saturation was only slightly reduced, despite a marked reduction of oxygen tension. Carbon dioxide content and carbon dioxide tension are elevated on the average up to 62.1 ± 7.5 and 50.8 ± 7.70 resp. The mean pH value is 7.349. In patients with a recompensated chronic pulmonary heart disease the average values for oxygen saturation are 89 ± 4.1 and for oxygen tension 67.7 ± 11.1. Carbon dioxide content and tension remain however on a statistically significantly higher level compared to compensated pulmonary heart disease, viz. Ca_{CO_2} = 72.0 ± 8.7 and Pa_{CO_2} = 65.6 ± 9.7. The values of pH *averaged 7.292 ± 0.016*. About 80 % of the patients included in this group have a noncompensated respiratory acidosis, which persists also in recompensated patients in the form of the so-called chronic respiratory acidosis. The haemoglobin dissociation curve shows in these patients a definite shift to the right which is due to Bohr's effect. Patients with chronic respiratory acidosis are subjectively in good health, which suggests that they have adapted themselves to chronic acidosis.

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The Effect of Acetylcholine Infusion on the Pulmonary Circulation in Cases of Impaired Ventilation

By

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The possible role of pulmonary vasoconstriction in the genesis of pulmonary hypertension is still a matter of debate. Fishman (4) has recently reviewed the literature concerning the effects of alveolar hypoxia, which is considered to be the most powerful physiological stimulus for pulmonary vasomotor activity. He concludes, mainly from experiments including acute hypoxia: "There is some evidence to support the idea of Liljestrand and von Euler that regional vasoconstriction in hypoventilated areas diverts the pulmonary blood flow to the well-ventilated portions of the lungs." However, the magnitude of this vasomotor activity is such that it is considered of minor importance when compared to the effects of passive cardio-respiratory influences in the normal pulmonary circulation.

Few studies have been performed in cases of pulmonary disease where the relative role of pulmonary vasoconstriction has been considered. Chudrey et al. (2) showed that infusion of acetylcholine changed the distribution of pulmonary blood flow in patients with chronic pulmonary emphysema. This effect was "best explained by presuming that acetylcholine dilated vessels constricted by hypoxia" but "this mechanism appears to be only one of several that determine the distribution of flow through emphysematous lungs."

Widimsky et al. (11) using Priscol were able to demonstrate a rather marked decrease of the pulmonary vascular resistance, possibly "due to vasodilatation in the periphery of the pulmonary arterial tree." However, they did not consider hypoxia as an important mediator of this vasoconstriction.

The present investigation was performed in order to study the effects of acetylcholine on the pulmonary circulation in patients with diseases affecting the distribution of inspired air, whether this was due to pulmonary disease or dysfunction of respiratory muscles.

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Methods

The techniques employed are similar to those described earlier (8) Pulmonary artery (PA) and pulmonary artery wedge (PCV) pressures were measured through a double lumen catheter on a six channel Elema Mingograph A second catheter was placed in the right atrium in 4 patients. An indwelling polythene catheter was placed in a brachial artery using the Seldinger technique for sampling of arterial blood and pressure measurements. Blood oxygen content was determined with a spectrophotometric technique. Expired air was analyzed in a micro-Scholander or a Haldane apparatus. Cardiac output was determined according to the Fick principle

Arterial carbon dioxide tension was measured as described earlier (1) using a modified Astrup technique. Oxygen tension in blood was calculated from pH and oxygen saturation using the Dill curve. Alveolar oxygen tension was calculated from the alveolar air equation assuming that alveolar and arterial CO_2 tensions are identical. This will lead to an underestimation of the alveolar O_2 tension if an alveolo-arterial CO_2 tension difference exists. The alveolo-arterial oxygen tension $(A-a)_{O_2}$ difference will be correspondingly underestimated and the figure given will be a minimum estimate.

Venous admixture to arterial blood (Qs/Qt) was calculated assuming a postcapillary saturation of 98 %. This assumption is not valid in cases with diffusion disturbances, but when only a change in Qs/Qt is looked for the error can be neglected (Qs/Qt will be over estimated and any change will be under estimated)

After a period of rest basal studies were made, followed by infusion of acetylcholine into the right atrium. In 4 cases this was done by means of a second catheter while in the other patients the double lumen catheter was withdrawn to a suitable position. The dosage was slowly increased until 5—10 mg/min. was given. The final dose was adjusted so as to have no effect on the systemic circulation and was maintained for at least 20 minutes. Pressure and blood flow measurements were repeated during the last 10 minutes.

Seven patients were then studied while performing exercise on a bicycle in the supine position. The work performed was approximately 100—200 kpm/min. which all patients were able to perform in a "steady state" as judged from heart rate and pressures. Sampling of blood and expired gas and pressure recordings were done between the 5th and 10th minute. A second period of acetylcholine infusion was then started while the patients were still exercising with the same dosage as at rest. Another set of data were obtained during the 15th and 20th minute of exercise.

Spirometry was performed with a modified Bernstein spirometer (Grimby & Söderholm, to be published)

Material

The cases studied have been selected to represent different types of ventilatory dysfunction All cases were referred to the laboratory for diagnostic or preoperative evaluation of cardio-pulmonary function. Vital statistics, pulmonary function and clinical data are summarized in table I.

Three cases (B. R E. R., A. M.) were included because of unilateral paresis of the diaphragm. In all cases fluoroscopy revealed a decreased mobility of the diaphragm on the left side and a high position in the chest. In case B. R. the case history indicated a duration of only 2 weeks, while E. R. had symptoms of dyspnoea since 3 months. In these cases electric stimulation of the phrenic nerve in the neck gave a normal response. The contraction of the paretic diaphragm was quite comparable to that of the contralateral side. Case A. M. had a history of 8 years and electric stimulation gave a feeble response on the left side but a normal contraction on the right side.

Bronchospirometry was not performed in these cases but it has been shown that exaeresis of the phrenic nerve causes a decrease of the oxygen uptake on the paretic side to 50—70 % of the calculated normal value and a decrease of the ventilation to 25—50 % of the calculated normal value (6, 9) We have assumed that a similar effect was present in these cases.

There was otherwise no indication of cardiopulmonary disease in any of these cases.

One case (A. T) had recurrent episodes of respiratory failure in connection with respiratory infections. She had been hospitalized

Table I. Spirometric and clinical data of the cases

Case	Sex	Age	VC Found	VC Expected	FEV1.0/VC Found	FEV1.0/VC Expected	MVVr Found	MVVr Expected	Clinical data
Group A									
B. R.	M	22	3.23	4.85	95	84	126	177	Temporary paresis of left hemidiaphragm following stab wound 2 weeks before study. Electric stimulation of the phrenic nerves gives equally good movements of both sides of the diaphragm
E. R.	M	59	2.74	4.46	72	70	71	125	Paresis of left hemidiaphragm of unknown aetiology. Markedly dyspnoeic since 3 months before study. Electric stimulation of the phrenic nerves gives equally good movements of both sides of the diaphragm
A. M.	M	57	3.83	4.45	72	78	79	158	Paresis of left hemidiaphragm following traumatic rupture 8 years earlier. Dyspnoeic. Electric stimulation of the phrenic nerves gives normal response on right side but feeble response on left side
Group B									
L. K.	F	34	—	—	—	—	—	—	Bronchial asthma
A. G.	M	59	3.37	4.05	47	70	57	131	Carcinoma of left upper lobe bronchus
N. E.	M	46	2.50	4.50	56	76	30	122	Bilaterally extensive bronchiectasis and chronic bronchitis
B. E.	M	61	2.77	4.35	47	68	33	124	Bronchiectasis within lingula and left lower lobe. Chronic bronchitis
A. T	F	68	0.57	2.89	71	73	18	58	Cyphoscoliosis since childhood
N. L	M	73	2.27	4.08	57	63	47	108	Pulmonary tuberculosis. "Destroyed lung" on left side

VC = vital capacity l. ATPS
FEV1.0/VC = forced expired volume in % of vital capacity
MVVr = maximum voluntary ventilation free frequency

Our thanks are due to Dr. G. Biörck who kindly helped us with the electrophrenic stimulations.

during such an episode with right heart failure but had recovered at the time of the study.

Two cases (N. E., B. E.) had histories of chronic bronchitis and bronchograms revealed bronchiectasis.

Case L. K. had history of bronchial asthma but was free of complaints at the time of the study.

Two cases had extensive parenchymal disease. Case A. G. had a bronchial carcinoma

Table II. Cardiac data before and during a constant infusion of acetylcholine at rest

Case		$\overline{P}_{PA}$	PCV	$\overline{P}_{BA}$	CO	TPR	PVR	SR	A-V O_2	V O_2
Group A										
B. R.	B	16	9	118	6.0	2.7	1.2	19.8	33	197
	D	14	8	112	6.7	2.1	0.9	16.8	32	214
E. R.	B	22	—	131	5.4	4.1	—	24.5	52	278
	D	17	—	133	4.6	3.7	—	28.6	47	218
A. M.	B	16	8	134	4.4	3.6	1.8	30.2	33	255
	D	14	8	133	4.6	3.1	1.3	29.0	49	225
Group B										
L. K.	B	10	7	82	6.5	1.6	—	13.1	29	182
	D	10	—	91	6.3	1.6	—	14.5	30	188
A. G.	B	13	5	105	4.6	2.8	1.8	23.0	42	192
	D	1[illegible]	4	100	4.6	2.6	1.8	22.0	47	214
N. E.	B	14	5	135	3.5	4.9	2.6	38.2	55	194
	D	11	7	126	2.7	4.1	1.5	47.1	84	228
B. E.	B	12	11	91	7.9	1.5	—	11.6	56	203
	D	13	—	100	8.3	1.8	—	12.0	57	307
A. T.	B	33	—	130	6.8	4.8	—	19.1	34	222
	D	22	—	147	6.2	3.6	—	23.8	36	222
N. L.	B	34	—	125	5.1	6.7	—	24.6	44	224
	D	35	—	125	5.4	6.5	—	22.8	42	227
Mean	B	18.9	7	116.8	5.5	3.5	1.82	22.7	42.0	224.1
	D	16.7	6	118.3	5.5	3.2	1.57	24.1	44.9	226.6
P values for diff. B—D		> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

B = before

D = during acetylcholine infusion.

$\overline{P}_{PA}$ = mean pulmonary artery pressure mm Hg

$\overline{P}_{BA}$ = mean brachial artery pressure mm Hg

PCV = pulmonary capillary venous pressure mm Hg

CO = cardiac output l/min

TPR = total pulmonary resistance $\left(\frac{\overline{P}_{PA}}{CO} \text{ mm Hg/l/min.}\right)$

PVR = pulmonary vascular resistance $\left(\frac{\overline{P}_{PA}-\overline{P}_{PCV}}{CO} \text{ mm Hg/l/min.}\right)$

SR = systemic resistance $\left(\frac{\overline{P}_{BA}}{CO} \text{ mm Hg/l/min.}\right)$

A-V O_2 = arterio-venous oxygen difference ml/100 ml.

V O_2 = oxygen uptake STPD ml/min.

Table III. Respiratory data before and during a constant infusion of acetylcholine at rest

Case		SaO_2	Pa_{CO_2}	$\dot{V}_A$	$\frac{V_D}{V_T}$	$\frac{Q_S}{Q_T}$	$(A-a)_{O}$
Group A							
R. R.	B	96.6	43	3.1	38.4	8.6	9
	D	95.7	39	4.1	33.0	11.6	27
E. R.	B	96.0	39	4.2	43.9	5.5	13
	D	91.9	37	4.1	40.0	20.3	36
A. M.	B	96.6	46	3.6	45.7	3.6	1
	D	94.3	43	3.4	44.4	10.9	16
Group B							
L. K.	B	94.0	38	3.4	36.5	9.1	18
	D	93.0	36	3.4	26.4	21.0	31
A. G.	B	90.5	37	3.5	48.1	20.7	44
	D	87.1	36	3.9	57.4	23.4	51
N. E.	B	94.8	49	2.9	62.6	9.8	12
	D	89.8	41	3.9	54.9	16.0	39
B. E.	B	86.0	45	4.7	53.6	36.8	44
	D	81.0	43	5.4	52.9	44.8	53
A. T.	B	91.8	57	2.8	54.3	27.8	14
	D	83.0	52	2.3	57.7	43.7	33
N. L.	B	81.9	52	2.8	52.3	20.1	13
	D	80.0	50	2.9	50.0	25.9	21
Mean	B	93.3	45	3.4	48.4	15.8	19
	D	88.5	42	3.7	46.3	24.4	34
P-values for diff. B—D		< 0.001	< 0.01	> 0.05	> 0.05	< 0.001	< 0.001

SaO_2 = arterial oxygen saturation %
Pa_{CO_2} = arterial carbon dioxide tension mm Hg.
$\dot{V}_A$ = alveolar ventilation l/min.
$\frac{V_D}{V}$ = dead space ventilation in per cent of total ventilation.
$\frac{Q_S}{Q_T}$ = venous admixture to arterial blood in per cent of cardiac output.
$(A-a)_{O_2}$ = alveolo-arterial oxygen tension difference mm Hg.

occluding the left upper lobe bronchus with atelectasis and case N. L. pulmonary tuberculosis with multiple cavities in the left lung. There was marked shrinking of the lung with displacement of the mediastinum to the left.

I the following presentation the cases with unilateral paresis of the diaphragm will be dealt with separately as group A, while the other patients are comprised in group B.

Results

The results obtained t heart catheterization are given in tables II—V

Group A

Spirometry showed in all cases some reduction in vital capacity but an al-

Table IV Cardiac data during exercise before and during a constant infusion of acetylcholine

Case		$\overline{P}_{PA}$	PCV	$\overline{P}_{BA}$	CO	TPR	PVR	SR	A V O_2	V O_2
Group A										
B. R.	B	28	12	128	11 4	2.5	1 40	11.2	80	912
	D	15	10	114	11 7	1.3	0.43	9.8	83	971
E. R.	B	58	—	146	12.3	4.7	—	11.9	81	934
	D	26	—	136	10.8	2.4	—	12.5	87	942
A. M.	B	21	11	132	6.9	3.1	1 45	19.1	94	647
	D	19	10	130	6.8	2.8	1.32	19.1	87	593
Group B										
A. G	B	20	8	131	6.4	3.1	1.88	20.5	75	473
	D	17	8	117	6.1	2.8	1 47	19.1	76	466
N E.	B	33	—	151	6.7	5.0	—	22.6	73	497
	D	18	—	147	7 1	2.5	—	20.7	68	489
B. E.	B	26	—	101	11.0	2.4	—	9.2	70	773
	D	17	—	104	11.3	1.5	—	9.2	70	791
N I	B	77	—	164	7.3	10.5	—	22.4	107	782
	D	74	—	180	(5.8)	—	—	—	98	(570)
Mean	B	31 0	10	131.5	9 1	3.5	1.58	15.8	78.8	715.3
	D	18.7	9	124.7	9.0	2.2	1 07	15.1	78.7	703.7
P values for diff B—D		< 0.05		> 0.05	> 0.05	< 0.02	—	> 0.05	> 0.05	> 0.05

Symbols, see table II

most normal ratio of the forced expired volume in 1 sec in relation to the vital capacity Maximum voluntary ventilation was markedly decreased in cases E. R. and A. M

Rest

Pulmonary artery pressure was increased in case E R The pulmonary capillary venous pressure (PCV) was normal. Cardiac output was normal or slightly decreased and the $(A\ V)_{O_2}$ difference exceeded 50 ml/l in 2 cases.

During acetylcholine infusion all cases showed a decrease in the pulmonary artery pressure without any consistent change in PCV pressure or cardiac output. The total pulmonary resistance decreased in all cases.

Arterial oxygen saturation was normal in all cases. Arterial CO_2 tension averaged 42 6 mm Hg and V_D/V_T 42.6 %.

During infusion there was some reduction in arterial oxygen saturation but the CO_2 tension and V_D/V_T rather decreased. In all cases the alveolo-arterial oxygen tension increased.

Exercise

During exercise all cases showed an increase in pulmonary artery pressure and case E. R reached a definitely pathological value The PCV pressure increased in both cases measured but was still with-

Table V. Respiratory data before and during a constant infusion of acetylcholine during exercise

Case		Sa_{O_2}	Pa_{CO_2}	V_A	$\frac{V_D}{V_T}$	$\frac{Q_s}{Q_T}$	$(A-a)_{O_2}$
Group A							
B. R.	B	94.9	40	19.1	18.5	7.8	30
	D	92.2	37	20.2	17.2	12.6	42
E. R.	B	93.7	37	19.9	24.1	9.0	23
	D	89.8	36	19.0	33.1	15.5	46
A. M.	B	96.7	43	11.3	27.9	3.1	6
	D	95.7	42	10.6	28.6	4.4	18
Group B							
A. G.	B	94.0	34	10.2	42.7	7.4	38
	D	91.3	34	9.8	50.0	11.6	58
K. E.	B	93.8	44	7.9	43.8	9.9	18
	D	92.7	47	7.9	51.5	12.6	22
B. E.	B	86.4	48	13.7	47.5	23.0	43
	D	71.0	48	12.5	48.8	42.6	55
K. L.	B	81.0	56	11.2	43.3	24.4	35
	D	73.8	68	(7.4)	(53.2)	—	—
Mean	B	93.2	41.0	13.7	34.0	9.9	27
	D	88.9	40.7	13.3	38.2	16.6	39
P-values for diff. B—D		> 0.05	> 0.05	> 0.05	> 0.05	< 0.05	< 0.01

Symbols, see table III.

in the normal range. Cardiac output was almost doubled.

During acetylcholin infusion the pulmonary artery pressure decreased in all cases. There was no significant change in PCV pressure or cardiac output. Total pulmonary resistance decreased to a lower level than at rest.

There was some reduction in arterial oxygen saturation during exercise. Arterial CO tension was lower than at rest and accordingly the V_D/V_T decreased.

During acetylcholine infusion there was some further decrease in arterial CO tension, no consistent change in V_D/V_T but arterial oxygen saturation decreased to the same extent as during infusion at rest (average 2.5 %). Case E. R. who had the most pronounced fall in pulmonary artery pressure also had the largest decrease in saturation (3.9 %).

Brachial artery pressure and systemic resistance were unchanged during infusion both at rest and during exercise.

GROUP B

Spirometry. No data are available in case L. K. All other cases showed a moderate to marked reduction in vital capacity and maximum voluntary ventilation. Case A. T. had a normal forced expirogram while the other cases had signs of obstructive pulmonary disease.

Rest

Pulmonary artery pressure was pathologically increased in 2 cases (A. T N I) The other 4 cases had normal pulmonary artery and PCV pressures. Cardiac output was normal or low and the $(A\ V)_O$ difference exceeded 50 ml/l in 1 case. Total pulmonary resistance exceeded 3 units in 3 cases.

There was no consistent change in pulmonary artery pressure or cardiac output during acetylcholine infusion. Case A. T who had a marked decrease in pulmonary artery pressure and TPR also had the largest fall in arterial oxygen saturation. Arterial oxygen saturation below 94 % was found in 4 cases and arterial CO_2 tension was below 45 mm Hg in only 2 cases V_D/V_T exceeded 50 % in 4 cases.

During infusion arterial oxygen saturation decreased in all cases and at the same time 5 of 6 cases showed a decrease in arterial CO tension. Alveolo-arterial oxygen tension increased as did the calculated amount of venous admixture.

Exercise

Four cases were studied during exercise and in all of them total pulmonary resistance increased, which indicates that the pulmonary artery pressure increased relatively more than the cardiac output.

Case N I had the most marked increase in pulmonary artery pressure and total pulmonary resistance and also a very marked decrease in arterial oxygen saturation. During acetylcholine infusion this case got an acute respiratory insufficiency with markedly increased CO tension in arterial blood and a decrease in arterial oxygen saturation to 75 8 % Total ventilation decreased, and the respiratory pressure variations in the pulmonary artery increased indicating an acute bronchoconstriction. In this case no steady state was obtained. It is to be noted that during this period of ventilatory insufficiency due to bronchoconstriction the arterial oxygen desaturation was of the same order as in case B. E. This case has been excluded from the calculations of means during exercise.

The other 3 cases showed a consistent decrease of pulmonary artery pressure during infusion and exercise without any change in cardiac output. Total pulmonary resistance decreased to values equal to or lower than those found at rest. All three patients had a decrease in arterial oxygen saturation but there was no change in arterial CO_2 tension in 2 cases and a slight increase in one. $(A\text{-}a)_{O_2}$ difference and calculated venous admixture increased.

Discussion

Group A. In these cases of unilateral paresis of the diaphragm no evidence was found of cardiopulmonary disease. The spirometric findings are in accordance with those described earlier (7) and our assumption of an altered distribution of the inspired air seems warranted. There are two major findings in this study to be discussed.

1 Decreased pulmonary resistance.

2. Decreased arterial oxygen saturation.

Both at rest and during exercise acetylcholine caused a drop in pulmonary artery pressure without any change in cardiac output or PCV pressure. Thus the total pulmonary resistance decreased (as well as pulmonary vascular resistance in both cases where it could be calculated) This finding is in contrast to that in normal man where no change in pulmonary

resistance has been found (5). As there was no change in brachial artery pressure or systemic resistance and only a minor change in PCV pressure, passive changes in the pulmonary vascular bed can be excluded. Thus a decreased vasomotor activity is the most probable explanation of this decreased resistance in the pulmonary circulation.

Arterial oxygen saturation was normal at rest. During exercise a slight decrease occurred in 2 cases, which however was less than the desaturation occurring during acetylcholine infusion both at rest and during exercise.

The possibility of induced bronchoconstriction and impaired ventilation during acetylcholine infusion must be considered as a potential cause of the arterial oxygen desaturation. We have assumed identity between alveolar and arterial CO_2 tensions when calculating V_D/V and V_A. The decrease in arterial P_{CO_2} which occurred both at rest and during exercise during acetylcholine infusion would indicate a considerable hyperventilation of some parts of the lung if an alveolo-arterial CO_2 gradient had been created (10). Such a gradient would lead to an underestimation of the calculated V_A and correspondingly increased V_D/V_T. However the changes in V_D/V and V were very small, and furthermore the alveolar ventilation per litre oxygen consumed remained constant. These findings definitely exclude induced alveolar hypoventilation as a cause of the arterial oxygen desaturation.

The respiratory pressure variations in the PCV or PA tracings did not change consistently which also speaks against an induced bronchoconstriction.

The decrease in arterial oxygen saturation during acetylcholine infusion can be expressed as an increased $(A\text{-}a)_{O_2}$ difference. In these cases with unilateral paresis of the diaphragm, the $(A\text{-}a)_O$ tension difference increased on the average from 8 to 26 mm Hg at rest and during exercise from 21 to 35 mm Hg. This increase could be due to an increased resistance to diffusion or to an increased venous admixture in arterial blood.

No studies have been made on the diffusing capacity in these cases, but preliminary results from this laboratory in another group of patients indicate that acetylcholine does not change the membrane component of the diffusing capacity for CO but possibly decreases the pulmonary capillary blood volume.

Thus an increased venous admixture in arterial blood (perfusion of poorly ventilated areas of the lung) is the most probable reason for this increase in $(A\text{-}a)_{O_2}$ tension difference.

No attempt has been made to separate the effects of a true shunt from the apparent shunt, which is due to uneven V_A/Q_C ratios. A rough estimate of the venous admixture was done assuming the oxygen saturation in the pulmonary capillaries to be 98 %. The validity of this assumption does not affect the conclusions drawn, since only a comparison is made before and during acetylcholine infusion in the same case. At rest the increase in venous admixture is equivalent to a "shunt" of 8 % of the cardiac output and during exercise of about 6 %. No relation was found between the amount of shunt and decrease in pulmonary artery pressure or TPR. No such relationship could be expected as acetylcholine presumably alters the distribution of V_A/Q_C ratios towards the mixed venous point ($V_A/Q_C = 0$).

In *group B* the clinical diagnosis varies but in all cases an unequal distribution of the ventilation-perfusion ratios might

be expected. In these cases acetylcholine infusion at rest gave a significant decrease in arterial oxygen saturation but the effect on the pulmonary artery pressure and the calculated total pulmonary resistance was less consistent.

During exercise, however pulmonary artery pressure, TPR and arterial oxygen saturation each decreased. The results are thus quite comparable to those discussed in group A.

Both group A and B have shown the most consistent results during exercise. To some extent this might be dependent upon extraneous influences which are eliminated during exercise, e. g. the relative variability in cardiac output before and during infusion is larger at rest than during exercise. It might also be dependent upon a more intense hypoxic stimulus during exercise. In group A a hemiparetic diaphragm and in group B marked signs of obstructive lung disease might well explain an increased unevenness of distribution of the inspired air during the increased demand of ventilatory effort. The very high V_D/V_T which did not decrease as ordinarily found during exercise lends further support to this theory.

These studies thus support the theory of v. Euler and Liljestrand (3) and are in full agreement with studies performed in mitral disease (8) as well as in cases of obstructive emphysema (2). It is concluded that regional hypoventilation is an effective stimulus to vasomotor activity and that in some cases at least, this can be of importance for the pulmonary hypertension which occurs during exercise.

Summary

Three cases of unilateral paresis of the diaphragm and six cases with pulmonary disease have been studied before and during acetylcholine infusion at rest and during exercise.

Arterial oxygen saturation decreased during infusion in all cases both at rest and during exercise.

Pulmonary artery pressure decreased during acetylcholine infusion in 6/9 cases at rest and in all cases studied during exercise without significant change in cardiac output or PCV pressure.

It is concluded that regional hypoventilation does induce vasoconstriction which is released during acetylcholine infusion.

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Clotting Tests and other Tests of the Haemostatic Mechanism in Cirrhosis of the Liver and their Diagnostic Significance

By

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Because of the important role played by the liver in the synthesis of the plasma proteins, investigations of changes in the plasma protein pattern are used in the diagnosis of liver dysfunction. Some of the blood coagulation factors are proteins with specific activities which can be relatively easily and rapidly analyzed. Quantitative analyses of blood coagulation factors have therefore been utilized as liver function tests. The following clotting factors are more or less decreased in parenchymatous diseases of the liver: factor I (fibrinogen), factor II (prothrombin), factor V (proaccelerin), factor VII (proconvertin), factor IX (Christmas factor) and factor X (Stuart factor) (1, 2, 3, 5, 6, 7, 8, 9, 12, 13, 14, 16, 18, 19, 21, 22). Factor VII, or factor VII complex (factor VII + factor X) has been found by most recent authors to diminish earlier and to a greater degree than the other clotting factors (1, 2, 5, 6, 8, 12, 23, 33). The thromboplastin generation test, reflecting the combined activity of factors V, VIII, IX, and X (+ PTA and the Hageman factor) has also been found to be a sensitive test of liver function (2, 8, 12, 19, 20). Proaccelerin, fibrinogen, and antithrombin III (serum antithrombin) are diminished only in cases of severe liver impairment (4, 5, 7, 8, 13, 16, 22, 23). In cirrhosis of the liver furthermore a spontaneous fibrinolytic activity in the blood has been reported to be a very constant finding (11, 15, 22).

The bleedings which occur in liver diseases have, however, a complex genesis. Not only is there an impairment of fibrin formation — there is also a defect in platelet function, i. e. a decreased number of circulating platelets and a prolonged bleeding time as well as defective vascular function, i. e. increased capillary fragility (22).

In this study an attempt was made to investigate:

1. The frequency of impairment of the haemostatic function in mild and severe cases of cirrhosis of the liver as established by some common methods.

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2 The relative sensitivity of these methods.

3 The correlation between the different tests of haemostatic function, i. e. if there was any need for more than one test.

4 The correlation between these tests and some other common liver function tests, i. e. if the tests on the haemostatic function gave information which we could not get means of the other tests.

Materials

The 34 patients in this study had histologically verified cirrhosis of the liver. They were divided into two groups — mild and severe cases. Cases with jaundice (or an icteric index according to Meulengracht which was higher than ten) or signs of portal hypertension, such as ascites and varices of the oesophagus at the time of investigation, were characterized as severe. The mild cases did not show these signs. Most of the patient who were characterized as mild cases were able to perform their daily work.

Methods

Alkaline phosphatases were determined according to Buch & Buch (25).

Bilirubin in plasma was determined as icteric index according to Meulengracht (24).

Bleeding time determination was performed by a modification of the method of Ivy and coworkers (37). The upper normal limit was 11 minutes.

Capillary fragility was determined by the application of suction cups (diameter 20 mm) to the supra spinatus regions, with 200 mm Hg for one minute. The test was considered to be pathological when more than ten petechiae appeared.

Cephalin time according to Waaler (34). The normal variation was obtained by testing 40 young normal subjects. Normal range with 95 % confidence 63—103 sec.

Collection of blood. Blood was collected from the antecubital vein with sharp and coarse, silicone-treated needles. The first 2—3 ml of blood were discarded, and then the blood was allowed to flow freely into the test tubes. The first portion of 5 ml was collected in a glass tube and incubated at + 37 C for exactly 3 hours. This tube had been treated with 10 % potassium hydroxide solution and had been rinsed with water. The two following portions were collected in lusteroid tubes. In one tube, containing 0.5 ml of a 2 % potassium oxalate solution, 4.5 ml of blood was collected, and in another tube, containing 1 ml of a 3 % trisodium citrate solution, 9 ml of blood was collected. Both tubes were centrifuged in an "International" refrigerated centrifuge, model PM 2 at 1 700 RCF and at + 4 C. The plasma was collected with silicone treated pipettes into silicone-treated glass tubes. The oxalated plasma was immediately adsorbed with barium sulphate (100 mg/ml). The plasmas were kept in an ice-bath until used.

Paper electrophoresis was performed according to Durrum, with a veronal buffer of pH 8.6 and an ionic strength of 0.075 (29, 30).

Platelet counting was performed according to Björkman (28). Values below 100,000/mm³ were considered to be below the lower normal limit.

Proaccelerin was determined by a modification of Aas method (35). The normal variation was obtained by testing 38 young, normal subjects. Values below the lower limit of the 95 % confidence interval were regarded as being decreased.

Prothrombin-proconvertin (PP) was determined according to Owren & Aas (36). The normal variation was obtained by testing the plasmas of 100 young normal subjects (50 women and 50 men). A dilution curve was made on every single plasma, and the mean dilution curve was constructed. When read on this mean curve, the "100 per cent values" of the normal plasmas were found to fall within the limits 67.5—132.5 % with 95 % confidence. Values below 67 % are considered pathological.

Prothrombin was also determined according to Quick, i. e. Quick's tromboplastin test (17). The normal variation was obtained by testing 40 young, normal subjects. Clotting times above the upper limit of the 95 % confidence interval were considered prolonged.

Thrombin generation test. Citrated plasma was diluted 1/20 with physiological saline

containing an isotonic veronal buffer of pH 7.3 and 0.5 % trisodium citrate. T the plasma dilution, lipid material to 0.06 mg/ml (31), and $CaCl_2$ to 0.025 M were added. After 5, 7 and 10 minutes, aliquots were re moved from the incubation mixture, blown down into fibrinogen and the clotting time was recorded. The test was performed at + 37° C. The variance of the test was deter mined on the basis of plasmas from 33 young normal subjects, and the 95 % confidence limits were calculated. With clotting times exceeding the confidence limits after 7 and 10 minutes, the test was considered pathological.

Thromboplastin generation test. The serum and the adsorbed oxalated plasma were diluted 1/20 with physiological saline, containing 1/5 its volume of an isotonic veronal buffer of pH 7.3. Into glass test tube 1 ml of the serum dilution, 1 ml of the diluted adsorbed oxalated plasma, and 1 ml of lipid material from human brain, 0.06 mg/ml (31) were pipetted, and the mixture was incubated at + 37° C for 6 minutes. Then 1 ml of previously warmed $CaCl_2$ solution, 0.025 M was blown down. After 3 7 10 15, and 20 minutes, aliquots of 0.2 ml were removed from this mixture and blown down into 0.2 ml normal human Dowex-50 treated plasma, and the clotting times were recorded. The variance of the test was determined on the basis of 50 normal plasmas, and the 95 % confidence limits were calculated. With clotting times exceeding the confidence limits after 5, 7 and 10 minutes the test was considered pathological.

Thymol flocculation test was performed ac cording to MacLagan and Lehmann (26, 27). The turbidity was indicated as the extinction ith filter 2 in 10 mm cuvette.

Results

The frequency of pathological esults in tests of haemostatic function is shown in table I

Among the severe cases, as the table indicates, one or other of th tests was pathological in 83 %, and in the group of mild cases in 68 %. Of the clotting tests, the PP-test seemed to be the most sensitive one, as it was positive at a higher frequency than the other tests in the group of mild cases. In this group only one case had a positive Quick s test. The same patient also had thrombin and thromboplastin generation tests which were positive. This mild case gave a clinical impression of being relatively severe, but showed none of the criteria of a severe case and was therefore grouped as mild.

On the other hand Quick s test was pathological at nearly the same frequency as the PP test in the group of severe cases. The thrombin and thromboplastin generation tests were positive in 5 cases in this group Of these 5 patients, 4 died within one year Thus, it seems as if these tests might have an ominous prognostic significance.

Thrombopenia was a frequent observa tion in the group of severe cases. In-

Table I Percentage of pathological results in tests of the haemostatic mechanism in mild and severe cases of cirrhosis of the liver

	Cases %				
	Mild		Severe		Total
PP test	27	(6/22)	58	(7/12)	38
Quick's test	4.5	(1/22)	50	(6/12)	20.6
Cephalin test	14	(3/22)	17	(2/12)	23.5
Prothrombin test	10	(2/19)	27	(3/11)	17
Thrombin generation test	4.5	(1/22)	50	(5/10)	19
Thromboplastin generation test	4.5	(1/22)	41.5	(5/12)	15
Bleeding time	48	(10/21)	9	(1/11)	34
Capillary fragility	30	(6/20)	37	(3/8)	32
Platelet counting	14	(3/22)	64	(7/11)	30
One or other of the tests pathological	68	(15/22)	83	(10/12)	74

The figures in brackets represent the number of pathological cases/number of cases investigated.

Table II The correlation between the different tests of haemostatic function

	No of patients with pathol. tests	PP test	Quick s test	Cephalin test	Pro accelerin test	Thrombin generation test	Thromboplastin generation test	Capillary fragility	Platelets	Bleeding time
		13	7	5	5	6	6	9	10	11
PP test	13	—	6		4	5	5	3	5	2
Quick s test	7	6	—	0	4	5	5	2	3	0
Cephalin test	5	2	0	—	0	1	1	3	3	3
Proaccelerin test	5	4	4	0	—	3	3	1	1	0
Thrombin generation test	6	5	5	1	3	—	6	2	3	0
Thromboplastin generation test	6	5	5	1	3	6	—	2	3	0
Capillary fragility	9	5	2	3	1	2	2	—	4	4
Platelets	10	5	3	3	1	3	3	4	—	4
Bleeding time	11	2	0	3	0	0	0	4	4	—

Table III Percentage of pathological values in routine laboratory tests in mild and severe cases of cirrhosis of the liver

	Cases		
	Mild	Severe	Total
	%	%	%
Icteric index	0	58 (7/12)	21
Thymol flocculation	27 (6/22)	75 (9/12)	44
Alkalin phosphatases	27 (6/22)	33 (4/12)	29
Electrophoretic changes	68 (15/22)	100 (12/12)	79
A/G quotient < 1.00	32 (7/22)	83 (10/12)	50
A/G quotient < 0.80	14 (3/22)	67 (8/12)	32

creased capillary fragility on the other hand was seen quite as often in the mild group as in the severe. The surprisingly high percentage of increased bleeding time in the group of mild cases was difficult to evaluate. In all these cases except one, the bleeding time exceeded the upper normal limit only by a few minutes

The correlation between the different tests of haemostatic function is shown in table II Only one of the cases with a normal PP value had a pathological Quick s test, thrombin and thromboplastin generation, and proaccelerin test. Of the clotting tests investigated, it therefore, seems to be sufficient to use only the PP test in the diagnosis of cirrhosis of the liver

On the other hand, there was less evidence of a correlation between the clotting tests and the other tests of haemostatic function. Thus, only 50 % of the cases with a pathological PP test had thrombopenia and vice versa. A similar relationship was seen between a pathological PP test and increased capillary fragility There was no correlation between increased bleeding time and other test of haemostatic function.

Thus, if the problem is to judge the bleeding tendency of a patient with cir

Table IV. The correlation between pathological results in tests of the haemostatic mechanism and those in routine liver-function tests in cirrhosis of the liver

	No. of patients with pathol. tests	PP test	Proconvertin test	Thromboplastingen. test	Platelets	One or other of the coag. tests pathol.
		13	5	6	10	25
Icteric index	7	6	2	3	4	6
Thymol flocculation	15	7	4	4	6	8
Alk. phosphatases	10	6	1	2	3	6
Electrophoretic changes	27	10	5	6	9	13
A/G quotient < 1.00	17	9	4	5	7	12
A/G quotient < 0.80	11	7	3	4	5	9

Left column: number of patients with pathological values in routine liver function tests. The other columns show how many of these patients had pathological values in tests of haemostatic function.

The upper horizontal figures show the number of patients with pathological values in tests of haemostatic function. The other horizontal figures show how many of these patients had pathological values in routine liver-function tests.

rhosis of the liver the bleeding time, capillary fragility and the number of platelets should also be determined.

The question of the possible correlation between clotting tests and the routine laboratory tests of liver function is illustrated in table IV. As will be seen, the correlation between the thymol flocculation test and the PP test is rather inconclusive as only about half of the patients with a positive PP test had a positive thymol flocculation test, and vice versa.

The correlation between pathological PP tests and changes in the electrophoretic pattern is also inconclusive. Of the 13 patients who had a pathological PP test, 10 had electrophoretic alterations, and of the ten cases with discrete electrophoretic changes, only one had a pathological PP test. However of the 17 cases with an A/G quotient of less than 1.00 9 also had a pathological PP test. Thus, as will be seen, the frequency of pathological PP tests, of other pathological clotting tests, and thrombopenia increases when there is a decreasing A/G quotient. All the six patients who died within one year after the investigation will be found among the 9 cases with a pathological PP test and an A/G quotient of less than 1.00. Thus a low A/G quotient and a high frequency of pathological clotting tests seem to characterize severe cases.

Discussion

The electrophoretic pattern was changed in 79 % of the cases with liver cirrhosis. It might, therefore seem as if this test would be sufficient, and perhaps make the other tests unnecessary. Changes in the electrophoretic pattern, however are rather unspecific, which makes it difficult to draw conclusions concerning a pathological finding and its relation to liver function.

For the diagnosis of partial impairment of liver function, no ideal laboratory

test is known The use of several tests is therefore necessary This study shows that the clotting tests are justified as a complement to other laboratory tests for the diagnosis of discrete impairment of the liver and for estimating the degree of impairment. The PP test seems particularly useful in the quantitative evaluation of liver damage, as the PP value generally decreases with increasing severity

Summary

Clotting tests and other tests of haemostatic function have been performed on 22 mild and 12 severe cases of cirrhosis of the liver in order to evaluate their diagnostic significance.

The PP test which was found to be the most sensitive one, was positive in 27 % of the mild cases and in 58 % of the severe. A positive thromboplastin generation test was found in 41 % of the severe cases. Of the 5 patients with a positive thromboplastin-generation test, 4 died within one year The test thus seems to have an ominous prognostic significance.

A decreased number of platelets was found in 64 % of the severe and in 14 % of the mild cases, discrete prolongation of the bleeding time in 34 % of all cases and increased capillary fragility in 32 % of all cases.

There was a certain correlation between a low albumin/globulin quotient and pathological clotting tests, but only an inconclusive correlation between the thymol flocculation test and the PP test.

The conclusion that can be drawn is that prothrombin proconvertin determination is justified as a complement to other laboratory tests in the diagnosis ... of the liver To judge the bleeding tendency of a patient with cirrhosis of the liver the bleeding time, capillary fragility and the number of platelets should also be determined.

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Iodine Metabolism in Endemic Goitre in the East of Finland with a Survey of Recent Data on Iodine Metabolism in Finland

By

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During the years 1954 to 1957 studies of the iodine metabolism in patients with endemic goitre were made on the Åland islands in the south-west of Finland (12) in Helsinki/Helsingfors on the south coast (29) and in Joensuu in the east of the country (4). Both in goitrous and in non-goitrous subjects in all of these regions the average urinary excretion from a test-dose of radioactive iodine was much lower than that reported from non-goitrous areas in the U. S. A. (5, 21) Holland (24) and Italy (1). On the Åland islands the urinary ^{131}I excretion was of the same magnitude as that found in central Sweden (13, 14) but towards the east of Finland progressively lower values were found. These results were similar to those obtained in goitrous regions in the Andes of Argentine (22) Venezuela (20) Holland (24) Italy (1) and Austria (2). From simultaneous determinations of the excretion of stable iodine in the urine the average daily iodine intake was estimated at 60—70 µg in the southwest of Finland and at 50—55 µg in the east (4, 12). At the same time Virkki (26) published extensive data on the iodine content and the average consumption of the various foods which make up the Finnish diet. In this way he calculated the daily iodine intake to be 70 µg in the south-west (Turku Åbo) but only 50 µg in central Finland (Kuopio).

In the early months of 1959 another study of endemic goitre was made in

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consider the value t 48 hours as the maximum thyroid uptake in the euthyroid, but not in the hyperthyroid subjects. In the latter group the uptake at 24 hours was also determined, and was used in the calculations as closer approximation to the maximum uptake. These measurements were mad with collimated scintillation counter at 30 cm distance from the surface of the neck. At this distance the aperture faced an area 15 cm in diameter. For backscatter correction was made by an empirical factor for the equipment used of 0.87. The standard, which was equal to the administered dose, was brought to olume of 50 ml, and measured at the same distance in air.

The serum protein-bound iodine (PBI) was determined by modification (11) of the method of Barker Humphrey and Soley. For the determination of the excretion of stable iodine in the urine, aliquots of the two 24-hour samples were treated as described before (12), and further analysed by the same method as for PBI. In the case of the urines it was again found that the final recovery may be 15—25 % too low. The values in the tables were not corrected for this loss. Many subjects were asked to bring sample of the milk they used, since according to Vilkki (26) milk is the largest single sourc of iodine in the Finnish diet. These samples were analysed for iodine in the same way as the urines.

Serum cholesterol was determined by a modification of Bloor' method based on the Liebermann-Burchard reaction.

The daily release of iodine from the thyroid (H) was calculated from the thyroidal uptake ^{131}I by the formula of Riggs (18, 22)

$$H = \frac{EU}{1 - U}$$

Alternatively it was derived from the total urinary excretion of ^{131}I by modification of that formula (18)

$$H_x = \frac{E\,(1 - E^x)}{E^x}$$

Here E = the daily excretion of iodine in the urine in µg,
U = the fractional uptake of ^{131}I by the thyroid (48 hours)
E^x = the fractional excretion of ^{131}I in the urine (48 hours)
H and H_x are expressed in µg

Results

The thyroidal uptake and urinary excretion of radioactive iodine

In all euthyroid groups (I—III) the mean thyroidal uptake of ^{131}I (48 hours) fell within a narrow range between 60.5 and 67.0 of the dose (table I). There were no significant differences between the groups. The mean thyroidal uptake (24 hours) in the hyperthyroid group (IV) was 66.9 of the dose and did not differ from that in any of the other groups.

The mean urinary excretion of radioactive iodine in 48 hours in the euthyroid groups (I—III) ranged from 27.8 to 35.0 of the dose, the highest value being found in the non-goitrous group. Both in the goitrous and in the non-goitrous subjects the use of iodized salt was associated with a slightly higher excretion of ^{131}I (tables I and II). None of these differences were however statistically significant. In the hyperthyroid group the mean urinary excretion in 48 hours, 13.7 % of the dose was significantly lower than in any other group ($p < < 0.001$).

The mean recovery of radioactive iodine, being the sum of the thyroidal uptake and the urinary excretion in 48 hours (U + E) was slightly lower in groups I and II (94.0 and 92.8 respectively) than in the non-goitrous group III (102.0). The difference from group III was significant both for group I ($p < 0.001$) and for group II ($p < 0.02$). The total recovery in the hyperthyroid group IV was only 79.6 %, which is significantly less than in any of the other groups ($p < 0.001$ to < 0.01).

The excretion of stable iodine in the urine

The excretion of stable iodine was determined in the large majority of sub-

the east of Finland, in the region of Savonlinna. The incidence of goitre in school children in this part of Finland varies from 15 to 30 %. The study was planned and carried out in close collaboration with the Medical Department of the Savonlinna Central Hospital and with the Department for Clinical Endocrinology, University Hospital, Leiden, Holland. The aim of this investigation was twofold. Firstly it was desired to establish the main features of iodine metabolism in yet another region of Finland, by making a screening study in a large number of subjects living there. Secondly detailed studies were to be made in a selected group of patients in order to explore some fundamental problems in endemic goitre about which existing information is scanty or conflicting. This applies to the rate of turnover of thyroid iodine, to the validity of the usual formulae for calculating the release of iodine from the thyroid and to the level of the daily faecal excretion of iodine in endemic goitre.

The present report deals with the former part of this investigation, the latter part will be published elsewhere (10).

Material and methods

Altogether 150 subjects were examined who had come to the Savonlinna Central Hospital for treatment, or had been referred there by local physicians who had been asked to collaborate in the investigation. For each subject a careful history was taken, including questions on the duration of goitre, its familial occurrence, consumption of milk and other features of the diet, the use of iodized salt, ingestion of iodine from other sources and treatment for thyroid disease. The histories made it clear that iodized salt was used very irregularly in many cases.

Thirty subjects were excluded because of recent use of iodine-containing remedies (cough-mixtures, iodine lotions for topical use etc.), previous X-ray examination with iodine containing contrast media, antithyroid therapy, thyroid treatment or previous thyroidectomy. The remaining 120 subjects were divided into four groups. A goitre was present in 96 subjects considered to be euthyroid, of whom 74 used iodized salt (group I) and 22 did not (group II). Group III contained 10 euthyroid subjects without demonstrable enlargement of the thyroid. Of these 6 used iodized salt and 4 did not. Separate data for these subgroups are given where this seemed relevant, but for many purposes group III was considered as a whole. Group IV consisted of 14 hyperthyroid patients.

The division into euthyroid and hyperthyroid cases was made mainly on clinical grounds, but the levels of serum cholesterol and PBI were used as supplementary evidence. On the basis of careful palpation of the neck, and when necessary X-ray examination of the tracheal area, goitres were divided into three classes of increasing size (12).

Of the 106 euthyroid subjects 12 were male and 94 female. All subjects were adults, the age distribution being 2 below 20, 52 between 20 and 40, 42 between 40 and 60 and 6 above 60 years of age. The distribution was similar for male and female subjects. According to thyroid size the 96 cases of goitre were distributed as follows: class I 65, class II 23 and class III 8 subjects. The majority of goitres were therefore of small size. Nodules were observed in all cases.

Of the 14 hyperthyroid patients 3 were male and 11 female. The age distribution was as follows: 3 were between 20 and 40, 5 between 40 and 60 and 6 above 60 years of age. In one patient the thyroid was of normal size, 7 belonged to class I, 5 to class II and 1 to class III.

Test-doses of 20 μC of radioactive iodine were given orally. The urinary excretion of ^{131}I was determined in two 24-hour samples collected during the two days following the dose, and the samples were also analysed for stable iodine. Urines were measured in a well-type scintillation counter. The thyroidal ^{131}I uptake was, for practical reasons, determined at 48 hours in the euthyroid subjects. On the basis of curves for the thyroidal ^{131}I uptake and retention, which were made in some of these cases, it seemed permissible to

Table II. The excretion of radioactive iodine in the urine in different parts of Finland

Locality and groups of subjects	No. of subjects	Urinary excretion of ^{131}I in 48 hours (% of dose)		References
		Mean	± S.E.	
Åland Islands				
Non-goitrous	27	49.3	2.3	Lamberg et al. 1958 (12)
Using I-salt	9	47.5	4.1	Lamberg et al. (unpublished)
No I-salt	18	49.5	3.7	Lamberg et al. (unpublished)
Goitrous	103	39.4	1.3	Lamberg et al. 1958 (12)
Using I-salt	49	42.5	1.8	Lamberg et al. (unpublished)
No I-salt	52	34.5	1.7	Lamberg et al. (unpublished)
Helsinki/Helsingfors				
Non-goitrous	27	45.2	2.1	Wahlberg et al. 1957 (29)
Goitrous	143	37.8	1.2	Wahlberg et al. 1957 (29)
Non-goitrous	28	44.3	—	Nikkilä and Karlsson 1960 (15)
Savonlinna				
Non-goitrous	10	35.0	4.0	Present study
Using I-salt	6	39.6	4.2	Present study
No I-salt	4	28.2	7.1	Present study
Goitrous				
Using I-salt	74	32.7	1.5	Present study
No I-salt	22	27.8	2.4	Present study
Joensuu				
Goitrous				
Using iodine	67	46.8	1.7	Jussila 1956 (4)
No iodine	145	*24.1	1.0	Jussila 1956 (4)

Not subdivided according to the use of iodized salt.
Using iodized salt and/or iodine in other forms.
24-hours excretion of radioactive iodine.

per day which differed significantly only from group II ($p < 0.01$).

The daily release of iodine from the thyroid (H)

In table I values for H obtained by both methods are given. The mean values calculated from the thyroid ^{131}I uptake (H_1) were slightly but not significantly higher in the goitre groups (I and II) than in the non-goitrous group III, the figures being 82.6, 80.3 and 69.4 µg. When, however, the urinary ^{131}I excretion was used to derive H_2, the discrepancy between groups I and II on the one hand, and group III on the other was much larger, since the values were 115.1, 113.5 and 64.5 µg. This difference was significant both for group I ($p < 0.001$) and for group II ($p < 0.02$). Both in group I and in group II the difference between H_1 and H_2 was statistically significant ($p < 0.01$).

Table I Data on iodine metabolism in goitrous, non-goitrous and hyperthyroid subjects in the endemic region of Savonlinna in the east of Finland

Group of subjects	U	E	U + E	E	H	H_1	PBI	Cholesterol
I Goitrous using I-salt								
Mean	60.5	32.7	94.0	47.8	82.6	115.1	6.28	[illegible]
S. E.	1.5	1.5	1 0	2.0	5.8	9.9	0.13	6.3
No.	68	74	68	67	64	67	71	[illegible]
II Goitrous no I salt								
Mean	65.6	27.8	92.8	37.8	80.3	113.5	6.00	[illegible]
S. E.	3.2	2.4	2.6	4.7	11.8	17 1	0.14	11.4
No.	17	22	17	17	12	17	22	22
III Non-goitrous								
Mean	67 0	35.0	102.0	34.9	69.2	64.5	6.17	296.2
S. E.	3.4	4 0	2.1	3.9	8.6	8.4	0.45	15.3
No.	10	10	10	10	10	10	4	4
IV Hyperthyroid								
Mean	66.9	13.7	79 6	59.1	–	–	13.0	202.9
S. E.	3.4	2 7	3.9	6.4	–	–	1 4	11.7
No.	14	14	14	14	–	–	14	14

U – thyroidal uptake of radioactive iodine in 48 hours, % of dose.
E – urinary excretion of radioactive iodine in 48 hours, % of dose.
U + E – total recovery of radioactive iodine in 48 hours.
E – urinary excretion of stabl odine, μg per day
H_1 – da ly release of iodine from the thyroid, calculated from thyroidal uptake, μg.
H_2 – daily release of iodine from the thyroid, calculated from urinary excretion, μg
PBI – serum protein-bound iodine, μg/100 ml.
Cholesterol – serum cholesterol, mg/100 ml.

[1] Not subdivided according to the use of iodized salt (see tables II and III)
24-hours uptake of radioactive iodine.

jects from groups I and II and in all from groups III and IV (tables I and III) The mean value was slightly but not significantly lower in the non goitrous group than in those with goitre Goitrous subjects who used iodized salt (group I) excreted more iodine than those who didn t (group II) the values being 47 8 and 37 8 μg per day A sim ilar difference was found within the non goitrous group III (40 3 and 23 0 μg per day) Neither difference was significant, but when all users of iodized salt in the three groups were compared with the non users, the mean values (47.2 and 34 0 μg per day) were significantly dif ferent ($p < 0.01$) In the hyperthyroid group the mean excretion was 59 1 μg

Discussion

From the data presented it appears that in all euthyroid groups studied in Savonlinna, the thyroid took up about two-thirds of a test dose of radioactive iodine. This is approximately double the thyroidal uptake found in goitre-free areas abroad (5 21 24) The urinary excretion of radioactive iodine found in Savonlinna was correspondingly lower than that in such areas.

The mean daily excretion of stable iodine in the urine of all euthyroid subjects (47.2 µg with the use of iodized salt and 34.9 µg without it) was much below the figures reported from goitre-free areas abroad, e.g. 182 µg found by Terpstra and Querido in Holland (25) Admittedly the present figures may be up to 25 % too low as indicated by recovery determinations. To estimate the average daily iodine intake (table III) the urine data were corrected for a loss of 25 % in the analysis. A value of 20 µg was assumed for the daily loss of iodine in the faeces, which according to indirect determinations (10) is generally below that amount. The mean iodine intake, which is probably overestimated in this way would be about 80 µg per day when iodized salt is used, and about 65 µg per day when it is not used. Both values, although not extremely low are clearly below the 100 µg limit, considered as the minimum human iodine intake in the WHO report on endemic goitre (23) Moreover the present study was made in late winter i.e. the season when, as Vilkki's findings in Turku and Kuopio seem to show (26) the iodine content of the milk is relatively high, probably because the cattle receive more iodine during indoor feeding than when grazing Since milk is a major factor in the human iodine supply in Finland, an even lower iodine intake might well have been found at other times of the year

Thus both the radioactive iodine data and the estimated iodine intake are in agreement with the conclusion that all of the euthyroid groups studied in Savonlinna were receiving too little iodine. Apparently the use of iodized salt, which had no effect on the thyroidal ^{131}I uptake, and only a minor one on the urinary excretion of stable iodine, did little to correct the shortage of iodine in the diet. It seems that the iodine content of this salt, or its consumption on the present voluntary basis, are too low to make this prophylaxis effective. When subjects with and without goitre were compared, the mean values of both the uptake of radioactive iodine and the excretion of stable iodine were remarkably similar in these groups. The implications of this fact, which has also been reported from Venezuela (20) are discussed below

Between the goitrous and non-goitrous groups there were, however discrepancies in the recovery of radioactive iodine and in the calculated release of iodine from the thyroid (H) but to some extent they appear to be related. The somewhat lower recovery in the goitre groups is most probably due to a relative underestimation of the thyroidal ^{131}I uptake, which may arise in two ways. Owing to the different geometry the ^{131}I uptake may be measured with a lower efficiency in goitre than in a normal-sized thyroid. A more rapid release of ^{131}I from the goitrous thyroid would also lower the measured thyroid uptake. The latter factor is certainly the one mainly responsible for the incomplete recovery of ^{131}I in the hyperthyroid group.

If this reasoning is correct, it will also explain why in the goitre groups lower

Table III The urinary excretion of stable iodine and the estimated daily iodine intake in different parts of Finland

Locality and groups of subjects	No. of subjects	Urinary excretion of iodine (μg/day) Mean	± S.E.	Estimated iodine intake (μg/day)	References
Åland islands					
Non-goitrous	27	49.1	4.1	—	Lamberg et al. 1958 (12)
Using I-salt	9	53.2	8.2	—	Lamberg et al. (unpublished)
No I-salt	18	42.6	4.5	—	Lamberg et al. (unpublished)
Goitrous	102	41.2	2.1	—	Lamberg et al. 1958 (12)
Using I-salt	49	46.0	1.0	—	Lamberg et al. (unpublished)
No I-salt	52	34.1	2.4	—	Lamberg et al. (unpublished)
Total using I-salt	58	47.1	2.9	80	Lamberg et al. (unpublished)
Total no I-salt	70	36.5	2.1	65	Lamberg et al. (unpublished)
Savonlinna					
Non-goitrous	10	34.9	3.9	—	Present study
Using I-salt	6	40.5	6.3	—	Present study
No I-salt	4	23.0	2.8	—	Present study
Goitrous					
Using I-salt	67	47.8	2.0	—	Present study
No I-salt	17	37.8	4.7	—	Present study
Total using I-salt	73	47.2	1.9	80	Present study
Total no I-salt	21	34.9	3.9	65	Present study
Joensuu					
Goitrous					
Using iodine	67	68.4	4.3	105	Jusila 1956 (4)
No iodine	145	28.1	0.7	55	Jusila 1956 (4)
Turku/Åbo	—	—	—	70	Vilkki 1956 (26)
Kuopio	—	—	—	50	Vilkki 1956 (26)

Values not corrected for losses in the analysis.
Using iodized salt and/or iodine in other forms.

Other data

The mean values of *serum protein-bound iodine* were well in the normal range in all euthyroid groups (table I). The mean levels of *serum cholesterol* in the euthyroid groups were almost equal (table I). According to Keys et al. (6) the level of serum cholesterol is higher in winter than in summer in inhabitants of eastern Finland (Karelia). When the present values, most of which were obtained in late winter, are compared with those found in Karelia in January, the similarity is close.

The mean iodine content of 85 samples of *milk* was 27.4 μg per litre (S.E. 2.2 μg), which is close to the annual average found by Vilkki in the region of Kuopio (26).

Discussion

From the data presented it appears that in all euthyroid groups studied in Savonlinna, the thyroid took up about two-thirds of a test dose of radioactive iodine. This is approximately double the thyroidal uptake found in goitre-free areas abroad (5, 21 24) The urinary excretion of radioactive iodine found in Savonlinna was correspondingly lower than that in such areas.

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Thus both the radioactive iodine data and the estimated iodine intake are in agreement with the conclusion that all of the euthyroid groups studied in Savonlinna were receiving too little iodine. Apparently the use of iodized salt, which had no effect on the thyroidal ¹³¹I uptake, and only a minor one on the urinary excretion of stable iodine did little to correct the shortage of iodine in the diet. It seems that the iodine content of this salt, or its consumption on the present voluntary basis, are too low to make this prophylaxis effective. When subjects with and without goitre were compared, the mean values of both the uptake of radioactive iodine and the excretion of stable iodine were remarkably similar in these groups. The implications of this fact, which has also been reported from Venezuela (20) are discussed below

Between the goitrous and non-goitrous groups there were, however discrepancies in the recovery of radioactive iodine and in the calculated release of iodine from the thyroid (H) but to some extent they appear to be related. The somewhat lower recovery in the goitre groups is most probably due to a *relative* underestimation of the thyroidal ¹³¹I uptake, which may arise in two ways. Owing to the different geometry the ¹³¹I uptake may be measured with a lower efficiency in goitre than in a normal-sized thyroid. A more rapid release of ¹³¹I from the goitrous thyroid would also lower the measured thyroid uptake. The latter factor is certainly the one mainly responsible for the incomplete recovery of ¹³¹I in the hyperthyroid group

If this reasoning is correct, it will also explain why in the goitre groups lower

values for H were obtained from the thyroidal ^{131}I uptake (H_1) than from the urinary excretion of ^{131}I (H_2). From Riggs' formula it may be seen that a small error in U will produce a much larger one in H_1, particularly when U is high. If U be 0.7 and is measured 10 % too low this will reduce the recovery by 7 % but H_1 by about 28 %, the differences observed being of that order (table I).

On the other hand H_2 does not depend upon U, and if errors resulting from incomplete collection of urine, or from the assumption that the ideal recovery is 100 %, would make H_2 too high, they would probably do so in all groups to the same degree.

Therefore the fact remains that H_2 was considerably higher in the goitrous than in the non-goitrous groups (a similar observation was reported previously (12)). This could indicate a real difference in the amount of thyroid hormone secreted per day but may also mean that, in this case at least, H gives a false estimate of hormone secretion. The latter possibility is supported by the observation that, in a number of these goitrous subjects, relatively large amounts of non-hormonal iodine were secreted by the thyroid, and of organic iodine were excreted in the urine (10). Both of these factors, which are incompatible with the model on which Riggs' formula is based, will cause an apparent increase of H. They may also explain part of the astonishing variation of H found in this and other (22) studies of endemic goitre.

For comparison the principal data from the several studies of iodine metabolism recently made in Finland, have been listed in tables II and III. The regions concerned were arranged in a sequence from the west of the country where the goitre incidence is relatively low to the east, where it is high. Clearly the results obtained in Savonlinna fit in with the previously noticed (12, 29) trend towards a lower urinary excretion (and therefore a higher thyroidal uptake) of *radioactive iodine* in the east of Finland (table II). This excretion was significantly lower in Savonlinna than in the Åland islands, both in goitrous patients who used iodized salt ($p < 0.001$) and in those who did not ($p < 0.05$). The excretion in the non-goitrous groups also seemed to be lower in Savonlinna than in the Åland islands, with an intermediate value for Helsinki. No statistical evaluation of these data is given, as the possible influence of iodized salt could not be eliminated. A comparison of the 24-hour excretion of ^{131}I in goitrous subjects not using iodized salt between Joensuu and Savonlinna yielded no significant difference.

The data on the excretion of *stable iodine*, all of which were obtained by the same method, show, at least in subjects not using iodized salt, a similar tendency to be lower in the east (table III). Only the non-users of iodized salt were considered in the statistical analysis. The iodine excretion was in goitrous patients lowest in Joensuu, and there was a significant difference between Joensuu and Savonlinna ($p < 0.001$) and between Joensuu and the Åland islands ($p < 0.01$) but not between Savonlinna and the Åland islands. When the non-goitrous subjects studied in Savonlinna and the Åland islands were included in this comparison, the result was virtually the same ($p < 0.05$ and $p < 0.001$ in the same order). By analysis of variance in all subjects not using iodized salt a significant decrease in iodine excretion

towards the east was again observed ($p < 0.001$) Without the use of additional iodine the daily iodine intake was estimated at about 65 µg both on the Åland islands and in Savonlinna and at 55 µg in Joensuu The difference in estimated iodine intake between west and east may be smaller than would be expected from the difference in goitre-incidence but the figures are admittedly crude and probably too high. These data from clinical studies agree reasonably well with those of Vilkki (26 table III) who gives a somewhat larger difference between Turku, wher the incidence of goitre is relatively low and Kuopio where it is high. Interestingly a difference in the iodine content of human milk was recently found between these same areas (8)

Thus it appears that the human iodine intake in many parts of Finland is no more than 50—70 µg per day the lowest values being found in some eastern regions.

These results were mainly obtained in goitrous subjects, but in a limited number of non-goitrous subjects similar values were found. On this level of iodine intake, which is clearly below the accepted minimum human requirement of 100 µg per day (23) a considerable effort by the thyroid is required to keep up hormone production. From Riggs formula it may be seen that the thyroidal ^{131}I uptake must be 30 % of the dose on an iodine intake of 150 µg per day 45 % on 100 µg and 63 on 50 µg per day Imperfections of the formula as mentioned above will only increase the required thyroid uptake on a given iodine intake. The thyroid iodine clearance will even increase by a factor 4 if the iodine intake falls from 150 to 50 µg per day The relation between iodine intake and thyroid ^{131}I uptake observed in Finland is reasonably close to that theoretically expected. It is hard to deny that the relatively high thyroid uptake required by the a ailable iodine supply and actually found in clinical studies, may be itself lead to an undue frequency of goitre in the population.

It is not claimed, however that lack of iodine is the only cause of endemic goitre in Finland (9) or elsewhere Constitutional factors may determine whether under given circumstances, the thyroid becomes enlarged or not. In no endemic region does the entire population become goitrous, and yet the affected and non-affected members do not seem to differ markedly in iodine intake or in thyroidal ^{131}I uptake, as found both in the present study and by Roche et al. (20) Even more striking is the observation that the level of the thyroidal ^{131}I uptake was extremely high among Indian tribes in Venezuela, in which goitre was rare (19) A predisposition towards goitre development may in some cases, be due to minor abnormalities of iodine metabolism, demonstrable by tracer techniques (10)

The possible relation between human goitre and the occurrence of positive goitrogens in the food, particularly in the milk, has recently received much attention in Finland. According to Virtanen and coworkers (3 7 27 28) no more than 0 1 % of the goitrogens ingested by cattle are passed into the milk, which therefore is unlikely to be an important source of goitrogens in man.[1]

[1] During the preparation of this manuscript Virtanen (Experientia 17 241 1961) published report on a large analysis of natural goitrogens in milk and vegetables in Finland, and concluded that these occur in such small quantities that they probably have no influence on human goitrogenesis.

values for H were obtained from the thyroidal ^{131}I uptake (H_1) than from the urinary excretion of ^{131}I (H_2). From Riggs' formula it may be seen that a small error in U will produce a much larger one in H_1, particularly when U is high. If U be 0.7 and is measured 10 % too low, this will reduce the recovery by 7 %, but H_1 by about 28 %, the differences observed being of that order (table I).

On the other hand H_2 does not depend upon U, and if errors resulting from incomplete collection of urine, or from the assumption that the ideal recovery is 100 %, would make H_2 too high, they would probably do so in all groups to the same degree.

Therefore the fact remains that H was considerably higher in the goitrous than in the non-goitrous groups (a similar observation was reported previously (12)). This could indicate a real difference in the amount of thyroid hormone secreted per day, but may also mean that, in this case at least, H gives a false estimate of hormone secretion. The latter possibility is supported by the observation that, in a number of these goitrous subjects, relatively large amounts of non-hormonal iodine were secreted by the thyroid, and of organic iodine were excreted in the urine (10). Both of these factors, which are incompatible with the model on which Riggs' formula is based, will cause an apparent increase of H. They may also explain part of the astonishing variation of H found in this and other (22) studies of endemic goitre.

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Finland. Most of the subjects were goitrous, but small non-goitrous and hyperthyroid groups were also studied.

The mean thyroidal uptake of radioactive iodine (48 hours) was almost equally high in all euthyroid groups, whether goitrous or not (60.5—67.0 % of the dose). The excretion of ^{131}I in the urine (48 hours) was correspondingly low (27.8—35.0 % of the dose). The ^{131}I data were not significantly influenced by the use of iodized salt. The hyperthyroid group was distinguished only by a lower urinary excretion of ^{131}I (15.7 % of the dose).

The mean urinary excretion of stable iodine in euthyroid subjects was 47.2 μg per day with the use of iodized salt and 34.9 μg per day without it. With corrections for incomplete recovery in the analysis of the urines and for the faecal excretion of iodine, the corresponding figures for the daily iodine intake were 80 and 65 μg.

These data suggest that in the population, and certainly in its goitrous members, the daily iodine intake is below physiological demands, and is little improved by the present voluntary iodine prophylaxis.

The estimated daily release of iodine from the thyroid (H) was, in most groups, found to be lower when calculated from the thyroidal uptake than when derived from the urinary excretion of ^{131}I. The latter values, which seemed more reliable, were considerably higher in the goitrous than in the non-goitrous subjects. The available evidence makes it doubtful whether this corresponds to a difference in the rate of thyroid hormone secretion between those groups.

From comparison of the present data with those obtained elsewhere in Finland, it appears that in large part of the country although most markedly in the east, iodine deficiency still prevails. The possibility that positive goitrogens in the diet may contribute to the goitre endemia, as some evidence seems to suggest, only increases the need for a more effective iodine prophylaxis.

Acknowledgements

Our thanks are due to Dr K. Sandelin, M. D., Head of the Savonlinna Central Hospital, for facilities at the hospital for carrying out the present study and to Miss E. Simell, of the Isotope Laboratory of the Savonlinna Central Hospital, for technical assistance. The statistical analysis was made by Mrs. E. Maronen, M. Sc., of the Statistical Bureau of the Institute for Occupational Diseases.

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Peltola (16) and Peltola and Krusius (17) however reported that rats receiving milk from a goitre area developed bigger thyroids than those which got milk from a goitre-free region even on an excessive iodine intake. They conclude that goitrogens "May be responsible for the human goitre endemia, especially as definite evidence of iodine deficiency in the food is still lacking" (17). This statement seems to be an oversimplification and the conclusion too far drawn, for the milk that promoted goitre came from one single dairy and no evidence about its effect on humans was given. On the other hand the criteria on which these authors tend to deny that in Finland iodine deficiency exists, and is related to human goitre, are certainly too rigid. As evidence for the importance of iodine deficiency in a given area it is not required nor even to be expected that PBI values should be lower in goitrous than in non-goitrous subjects, nor that these groups will differ markedly in iodine excretion or in thyroidal ^{131}I uptake. Therefore the fact that such differences were actually not found by no means allows the conclusion that an iodine intake much below 100 μg per day, which according to the data cited by Peltola and Krusius (16, 17) is usual in Finland, is adequate.

From a practical point of view there is hardly any place for a controversy between an iodine deficiency and a goitrogen theory, for it is widely agreed that the effect of dietary goitrogens will be aggravated by want of iodine. This is self-evident for substances with the thiocyanate type of action, but it should be realized that the late effect of thiouracil-like substances is mainly iodine depletion from which recovery will be more difficult on a low iodine diet. In other words, if the ingestion of goitrogens is at all important the iodine intake must be above the minimum of 100 μg per day to give the attainable protection against the development of goitre. Clearly this requirement is not met by the natural Finnish diet, nor is it reached by the voluntary use of iodized salt, which apparently adds no more than 15 μg to the daily iodine intake, and brings it to 80 μg at the utmost. The seemingly larger effect of additional iodine observed in Joensuu (table III) is almost certainly due to the use of iodine in forms other than iodized salt.

A similar experience was reported by Terpstra and Querido (25) who, in an endemic area in Holland found that the voluntary use of iodized salt (5 mg/kg) had no perceptible influence on iodine excretion or on thyroidal ^{131}I uptake. The compulsatory use of iodized salt (40 mg/kg) by bakers, however brought these parameters of iodine metabolism to values found in goitre-free regions, and greatly diminished the incidence of goitre.

In conclusion one may say that studies of human iodine metabolism, in regions of Finland ranging from the south-west to the east, have, in spite of the present prophylactic measures, consistently demonstrated the features of iodine deficiency. The largely theoretical possibility that natural goitrogens may contribute to the occurrence of endemic goitre in Finland should be no reason to tolerate insufficient iodine supply. On the contrary it only serves to emphasize the need for a more effective iodine prophylaxis.

Summary

Studies on iodine metabolism were made in 120 subjects living in an endemic goitre area (Savonlinna) in the east of

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Noradrenaline — Adrenaline Producing Nonchromaffin Paraganglioma[1]

By

Peter Berdal, Magnus Braaten, Chr. Cappelen Jr,
Erling A. Mylius and Otto Walaas

The so-called glomus tumours or nonchromaffin paragangliomas (18) are rare lesions originating from small cell formations, the nature and function of which are as yet little understood. Detailed information with regard to these structures was first given by Luschka in 1862 (23). He described the anatomy and histological structure of the carotid body, which was first mentioned by von Haller in 1743 (12) as the ganglion minutum.

In 1840 Valentin (30) described a ganglion-like formation at the initial part of the tympanic nerve, and Krause (1878 (16)) demonstrated a formation in the initial part of the canaliculus tympanicus which had the same histological structure as the carotid body. From Guild (1941 and 1953 (10, 11)) we have a comprehensive description of the glomus jugulare. He demonstrated small formations of the same structure as the carotid body disseminated in the adventitia of the dome of the jugular bulb and along the initial part of Jacobson's nerve (tympanic branch of the glossopharyngeal nerve) and Arnold's nerve (auricular branch of the vagus) and in the middle ear. The term "nonchromaffin paraganglia" is suggested by Watzka (1943 (31)) for these normal structures. The term "nonchromaffin paraganglioma" is suggested by Lattes & Waltner (1949 (18)) for tumours arising from any member of this group of organs and is adopted by Guild (1953 (11)), Arnvig (1957 (1)), Lederer et al. (1958 (21)), Taylor (1958 (28)) a. o.

The glomus bodies are normally found as small ovoid, pale tan, formations 0.1—5 mm in size located mostly close to the point of ramification of the large vessels in the mediastinum (aortic bodies) and in the neck (carotid bodies). They

[1] A preliminary report was given before the Norwegian Oto-Laryngological Society Nov. 26th 1960.

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and one in which there is more or less faithful reproduction of the normal structure. The second or *adenoma-like* type in which the tumour cells have a pronounced epithelial appearance with rounded or polyhedral shape, abundant cytoplasm, and arrangement in sheets or rows. The third type may be called *angioma-like* Here the cells are largely spindle-shaped or crescentic and appar ently closely related to capillaries. In spite of these variations, however the fundamental pattern appears to be the same.

Tumours originating from glomus tissue in the jugular bulb, in the middle ear and in the Fallopian canal, may be seen in the middle ear as a bright red, or bluish red, lobulated growth behind the drum which may show evidence of pressure from the tumour If the tumour has penetrated into the uditory canal haemorrhages may readily occur Visible pulsation of the tumour upon light compression is a typical feature. The tumours developing in the middle ear are accompanied by hearing loss and tinnitus, the latter appearing synchronously with the pulse. There may be otorrhoea, but this is due to secondary inflammation. Paresis of the facial nerve is not uncommon. The tumour may invade the temporal bone and grow intracranially presenting exten sive neurological symptoms. Metastases in the lungs, the liver the bones and lymph nodes have been reported but are rare.

It is generally accepted that radical surgery wherever possible, is the best treatment. These tumours have very little sensitivity to radiation, and radiological treatment therefore is of questionable value.

The following case of a carotid body tumour recently bserved at the E. N T department of the University Hospital, Oslo may shed new light on the question of a possible secretory activity of the tumour cells, hormone-like in character

The patient was a 66-year-old man who had been ware of a large lump on the right side of his neck for about year prior to the first examination, which took place in Sept. 1960. At that time a protruding tumour the size of a tangerine, was observed on the right side of the neck located beneath the middle section of the sternocleido mastoid muscle (fig 1) The tumour was protruding into the pharynx and could be felt as an ovoid, well defined tumour with smooth surface and soft elastic consistency Carotid angiography disclosed that the external and internal carotid arteries had been forced part by the neoplasm (fig. 2) Routine clinical and labora tory examination revealed nothing abnormal except for slight emaciation. His blood pressure was 120/80 mm Hg. A routine endocrinolog ical examination gave negative results (see later). At operation in Oct. 1960 the tumour was found t be well defined, capsulated and had numerous large vessels on the surface ex tending into the surrounding tissues. The tumour was situated behind the carotid artery extending both bove and below the bifurcation and forcing the external and internal carotid arteries apart. It was separated from the carotid artery by its capsule.

The carotid arteries themselves appeared to be normal. The weight of the tumour was about 70 g Fig. 3. demonstrates the macroscopic appearance of the tumour

Following the operation the patient developed bulbar paresis on the right side with paralysis of the soft palate, pharynx muscles, the right side of the tongue and the right vocal cord. In consequence the patient had to be fed with the aid of stomach tube. Post operatively he was weak and showed lack of initiative, but was up and about. The bulbar paresis persisted. The patient died on May 7th 1961 from lobar pneumonia accompanied by acute severe diarrhoea.

During the operation the tumour was manipulated and compressed for 2 to 3 min. four times at intervals of about 10 min. and the intralumen arterial pressure was registered. During and immediately following this pro-

may also be closely related to nerves and vessels in the upper part of the neck and in the middle ear (glomus intravagale and juxtavagale, glomus jugulare, glomus tympanicum)

Histological findings show the glomus tissue arranged in more or less distinct lobules with nests of epitheloid cells surrounded by connective tissue with collagen and reticulum fibres and numerous capillaries. There is also an abundance of nerve fibres.

It has been widely discussed whether the glomus cells are of mesodermal or ectodermal origin. Embryologic studies by Boyd (1937 (2)) Hammond (1941 (13)) and others indicate the existence of a close relationship between glomus caroticum, glomus jugulare and glomus aorticum on the one hand and the vagal and the sympathetic nerves on the other. The close relationship between these bodies and the vagal ganglion has been emphasized in particular. Some investigators point out that occasionally ganglion cells are found closely intermingled with nerve fibres and that such elements are also found in tumours (Lattes 1950 (17))

The function of these structures is still not understood

According to Heymans and Bouckaert (1939 (14)) Schmidt and Comroe (1940 (26)) and Dripps and Comroe (1944 (5)) they are chemoreceptors sensitive to changes in the pH the carbon dioxide and oxygen tension of the circulating blood and under certain conditions they may be of major importance in the regulation of respiration

A possible adrenaline production has been discussed by several authors, but has never been demonstrated (3 4 17 29)

Recently Lever et al. (1959 (22)) reported that "The results of the various histochemical tests strongly suggest the presence within the glomus cell cytoplasm of a phenolic amine or some closely related chemical substance" This observation indicates a functional similarity between the glomus cells and the cells of the adrenal medulla.

The first report of a glomus tumour is attributed to Marchand (1891 (24)) who described a tumour of the carotid body. Rosenwasser (1945 (25)) reported the first case of a glomus jugulare tumour. Since that time numerous cases of tumours originating in the glomus caroticum and glomus jugulare have been reported, and even the aortic bodies (Lattes 1950 (17)) have been found to be the origin of similar neoplasms.

Nonchromaffin paragangliomas are generally met with in adults aged 30—70 years old the average age being around the middle forties. Such tumours have also been described in babies and in persons over eighty

Tumours originating in the tympanic and jugular paraganglions and developing in the middle ear are mostly found in women (32) Carotid body tumours, however do not display such a sex difference (27) These tumours grow slowly Case histories of 8—10 years are not uncommon. They are usually not malignant neoplasms in the general sense but owing to their site and invasive growth they may be serious to the patient. In some cases their complete removal is difficult and there may be a recurrence. The microscopical structure of these tumours is very similar to that of the normal glomus. For descriptive purposes Le Compte (1948 (19)) has subdivided the tumours into three groups The first or *usual* type is the most frequent variety

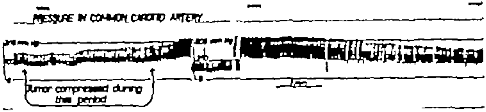


Fig. 4. Pressure curve from the common carotid artery registered through 22 gauge needle inserted in the lumen approximately 5 cm below the carotid bifurcation. The needle was mounted directly on an inductive type transducer The demodulated and amplified signal was fed to direct writing recorder

In the central part of the curve the sensitivity is temporarily changed from 200 mg Hg to 500 mm Hg full scale deflection owing to the high rise in pressure.

The presented curve is the third out of four different curves recorded under similar circumstances. The other three curves showed the same pattern, but the maximum rise was somewhat lower



Fig. 5. Sheets of large polyhedral cells with pale cytoplasm and round large nuclei. The septa of connective tissue are loose and highly vascular Zenker-fixed H. E. S. 250.



Fig. 6 Tumour nodules and capillaries in the wall of large vessel. Zenker-fixed H. E. S. 250



Fig 1 Lateral view of th patient s neck (see text)



Fig 2. Carotid angiogram demonstrating the broad angl between the internal and th external carotid artery



Fig 3 Photography of the tumour The surface was smooth, slightly lobulated. The cut surface dark red or grey

cedure a marked rise in the systolic and diastolic pressure was observed. From a level of 140 mm Hg the systolic pressure increased in the course of 3 to 4 min. to 210—220 mm Hg — on one occasion to as much as 250 mm Hg (fig 4) When the compression of the tumour was stopped, the systolic blood pressure returned to original values in about 5 min. The changes in the diastolic pressure were less pronounced and of shorter duration, increasing from 80 mm Hg to a maximum value of 120 mm Hg followed by a return to the initial value which occurred 2 min. prior to the return of the systolic pressure During the pressure period the pulse rate was accelerated The presented curve is the third in a series of four different curves which were recorded during the operation. All curves showed the same general pattern, but the maximum rise n blood pressure was somewhat lower in the others.

Histological examination of the tumour shows a highly cellular neoplastic tissue surrounded by a connecti e tissue capsule with isolated and well developed nerve fibres and occasionally large ganglion cells. Some of the nerve fibres are myelinated, others are not. The tumour tissue is mainly composed of quite large polygonal cells arranged in clusters

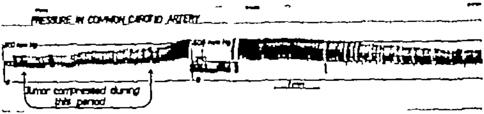


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Fig. 6. Tumour nodules and capillaries in the wall of a large vessel. Zenker-fixed H. E. S. × 250.



Fig 7 Some of the tumour cells have more or less Nissl-like granules in the cytoplasm. Zenker fixed, gallocyanin stain × 640

giving the tumour a lobular structure. The lobules are separated by slender strands of connective tissue with numerous dilated capillaries partly filled with blood (fig 5 and 6) The tumour cells are rather uniform with indistinct outlines. The cytoplasm is eosinophilic, rahter light in colour and is moderately rich in fine granules giving a light brown appearance. The nuclei are round, rather even in size with fine chromatin markings.

Occasional mitoses are found. The cellular picture resembles to some extent that of phaeochromocytoma, but unstained slides shows no chromaffinity

Certain areas of the tumour tissue shows a mixture of epithelial cells arranged around blood vessels, and bundles of nerve fibres. In some places there are also groups of larger cells among the glomus cells and some of them have the appearance of ganglion cells (fig 7) The histological findings are typical of nonchromaffin paraganglioma (carotid body tumour) We were curious to know whether or not biologically active amines could be detected in the tumour especially adrenaline, noradrenaline, serotonin, histamine and acetylcholine. The following analyses have been performed on samples from the tumour tissue *adrenaline and noradrenaline assays* at the Institute for Medical Biochemistry and Physiology University of Oslo, and adrenaline, noradrenaline, normetanephrine, dopamine and serotonin assays at the Institute of Pharmacology, University of Gothenburg.

Analyses for catecholamines in the 24 hr urine samples were carried out by an unpublished modification of von Euler and Floding's fluorimetric trihydroxyindole method (1956) using a Hilger spectrofluorometer Prior to the operation there was a markedly elevated urinary noradrenaline elimination of 430 μg in 24 hours (the normal values being 10—60 μg) On the other hand the adrenaline elimination was not definitely elevated. The amount was 18 μg in 24 hours, the normal values being 1—15 μg Twenty-four hours after the operation the urine showed normal catecholamine values.

Assays of the catecholamines in the tumour tissue were performed following extraction with acid ethanol (2 parts 96 % ethanol to which was added 0.25 ml 10 N H_2SO_4 per 100 ml ethanol) in a Waring Blender After centrifugation the ethanol was evaporated under vacuum at low temperature. The lipid fraction was removed by ether extraction. The ether was removed by evaporation after the pH had been adjusted to 3. The catecholamines were determined by the same method as described for the urine. These analyses showed that this specimen of the tumour contained considerable amounts of noradrenaline, 550 $\mu g/g$ The adrenaline content was 38 $\mu g/g$ tumour tissue. Normal content of noradrenaline in the suprarenal medulla is about 0 09 mg/g wet tissue and adrenaline 0 5 mg/g wet tissue (9) Serotonin could not be detected in the tumour The results of the amine assays in another specimen of the tumour tissue carried out at the Institute of Pharmacology University of Gothenburg, were as follows Adrenaline 10 $\mu g/g$ tumour tissue, noradrenalin 2,100 μg normetanephrin 7 μg metanephrin 0, dopamine 5 μg Serotonin 0 and histamine 0.4 μg

Results

Since it has been generally assumed that the nonchromaffin paragangliomas have no internal secretory function, it is of interest to note that the tumour in this case did contain catecholamines, in particular large amounts of noradrenaline. Previous observations which may indicate adrenaline production by these tumours were made by Le Compte (1948 (19)) and Terracol et al. (1956 (29)). Le Compte examined two cases of carotid body tumour with respect to adrenaline. The fresh tissue was frozen 2—3 hours after the operation and was extracted with 0.01 N hydrochloric acid. This extract was compared with an adrenaline solution of known concentration by comparing the effect on blood pressure in spinal cats and on isolated strips of ileum in the rabbit. One of the tumours showed an activity corresponding to less than 8 µg/g adrenaline tumour tissue, i. e. a negligible amount.

The second tumour had an adrenaline like effect corresponding to approximately 0.4 mg/g according to the blood pressure test, and 0.23 mg according to the ileum strip test. Further examination of this tumour indicated, however, that the pressor substance in question was probably not adrenaline.

Terracol demonstrated a rise in blood pressure in one case in connection with the removal of a glomus tumour located in the ear (increase from 125/70 to 165/100). In another case, operated on under hypothermia, the blood pressure fell during the anaesthesia to 90/60 and then increased to 120 when pressure was applied on the tumour.

It would appear possible that these observations of Le Compte and Terracol could be taken as evidence for catechol amine production in these tumours. It should also be noted that Engel and von Euler (1950 (6)) dignosed a case of phaeocromocytoma located at the bifurcation of the abdominal aorta by detecting increased urinary elimination of noradrenaline. The tumour itself also contained large amounts of noradrenaline.

In the present case a high catecholamine content was demonstrated in the tumour with the aid of specific fluorophotometric methods of analysis. It is highly probable that the catecholamines were produced by the tumour cells. This is indicated by the high urinary elimination of noradrenaline present preoperatively but ceasing simultaneously with the removal of the tumour. A predominant noradrenaline elimination in the urine is according to von Euler & Floding (8) a characteristic feature of chromaffin-cell tumours outside the adrenal medulla, while tumours originating from the adrenal medulla itself usually produce large amounts of both adrenaline and noradrenaline. The histological findings in this case closely resemble those usually observed in pheochromocytomas. The tumour has a distinct internal secretory appearance with groups of cells arranged close to blood vessels, the walls of which are very thin. However there are no chromaffin cells. From a purely morphological point of view this carotid body tumour resembles to a considerable extent the pheochromocytoma which develops from the adrenal medulla and, therefore, has a histogenetic basis in the nervous system and is thus of ectodermal origin. Although the nonchromaffin paragangliomas may present a varied histological picture it is noted in all reports that the tumour tissue is *nonchromaffin*.

This study has possibly added a new feature to the nonchromaffin paragangliomas, in that large amounts of noradrenaline have been demonstrated in the tumour cells. This brings out similarities between the pheochromocytomas and nonchromaffin paragangliomas both morphologically and functionally. The suggestion of a production of phenolic amines normally in the carotid body cells (22) is fully in accordance with the present finding. As to the histogenesis there now appears to be an even better foundation for the assumption that the tumour cells in the nonchromaffin paragangliomas are of ectodermal origin with epithelial appearance and endocrine function.

Hunt (1961 (15)) describes a case of a large carotid body tumour on the right side. The patient suffered from severe acute attacks of abdominal pain and diarrhoea. These symptoms disappeared when the tumour was removed. The patient's blood pressure was normal and there was no catecholamine elimination in the urine. It was assumed that the tumour had produced an unknown substance which had the above mentioned effect on the intestines.

Summary

The literature pertaining to glomus tumours or nonchromaffin paragangliomas is briefly reviewed and a case of carotid body tumour located in the neck of a 66-year-old man is described. The tumour which had the size of a tangerine had developed in the course of a year. The 24-hour urinary elimination of noradrenaline was markedly increased (430 µg) while the adrenaline elimination was not definitely increased. Twenty four hours after the operation the catecholamine values in the urine were normal. Analyses of two specimens taken from the tumour showed noradrenaline values of 550 and 2 000 µg/g, whereas the adrenaline values were only 33 and 10 µg/g in the same specimens. Following manipulation and compression of the tumour during the operation a brief rise in systolic blood pressure from 140 mm Hg to 210—250 Hg was recorded. A rise in the pulse rate was registered as well. Histologically the tumour tissue was composed of large polyhedral nonchromaffin cells and had morphologically similarity to pheochromocytoma. The histological pattern and the recognition of an internal secretory activity with production of noradrenaline may point to a histogenetic relation to the nervous system, and therefore we held that the carotid body tumour has an ectodermal oncogenesis.

Acknowledgements

The catecholamine analyses which were performed at the University of Gothenburg, were carried out under the supervision of professor A. Carlsson M. D. by Dr J. Häggendal and Miss Margit Lindquist, and by dr M. Westling. We express our grateful thanks.

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Addendum

Since submission for publication case of 1-nor adrenaline-producing tumour in the neck has been reported by GLENNER, G. G., CROUT J R. & ROBERTS, W C. A functional carotid-body-like tumor A. M. A. Arch. Path. 73 230 1962.

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Long term Treatment of Chronic Pyelonephritis

By

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The successful treatment of chronic pyelonephritis is one of the most difficult problems associated with renal disease. It is of great importance, however, that the difficulties be mastered since chronic pyelonephritis is the most common of the kidney diseases observed at autopsy (2, 13, 22) being found, according to many investigators, in approximately 6—9 per cent of autopsied cases (2, 10, 16). Only in about one-sixth of these cases had pyelonephritis been diagnosed before death (16). Chronic pyelonephritis was considered to have been the principal cause of death in about one-third of the cases in which it was observed (10).

Chemotherapeutic agents and antibiotics which ameliorate acute attacks do not appear to have any major influence on the progress of the disease and on the autopsy statistics (10). Until recently the conventional treatment has consisted of short-term high-dosage therapy which, as a rule, suppresses the acute symptoms. Certain recent reports (6, 8, 11, 18, 20) as well as experience at this clinic indicate that chronic recurrent infections can usually be prevented by prompt and adequate treatment of any acute infection occurring in an unobstructed urinary tract.

Chronic pyelonephritis may stem from a persistent infection in apparently normal kidneys, or it may be due to a renal *locus minoris resistentiae* which provides an ingress for recurrent infections. A minor anatomic anomaly or a scar from a previous acute kidney infection may constitute such a *locus minoris resistentiae* (2, 15).

Our best results in the treatment of chronic pyelonephritis have been achieved by not confining ourselves to subduing the recurrent urinary infections but by carrying out continuous long term therapy with sulfonamides and/or nitrofurantoin. During the last years penicillin has also been used in long-term treatment.

The diagnosis of chronic pyelonephritis may present difficulties, particularly since the relapses are frequently asymptomatic.

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Table I Incidence of some principal symptoms of chronic pyelonephritis prior to treatment of 36 female patients

	No. of cases	%
Periodic febrile cystitis	24	67
Periodic afebrile cystitis	4	11
Headache	23	64
Backache	22	61
Hematuria	7	19

However our possibilities of diagnosing the disease have been enhanced by a growing knowledge of the often quite diffuse subjective symptoms by the more extensive use of renal function tests and by early detection of asymptomatic bacteriuria (1 3 13) Beside these tests it is important that each case is followed by frequent and careful examinations of the urine and the blood

Long term treatment of chronic pyelonephritis has been recommended by several authors. In 1954 Stansfeld and Webb (18) described a therapeutic regimen for children with chronic pyelonephritis. The treatment began with a relatively high dosage of sulfadimidine or an antibiotic for a two-week period, followed by a reduced dose of sulfadimidine over a period of two to six months. Two similar groups of cases were compared — one treated in the usual manner and the other in accordance with the new regimen. The superiority of the long term treatment was demonstrated.

A similar therapeutic regimen was reported by Taylor in 1955 (20) Administration of tetracycline for short periods was followed by intermittent long term therapy with sulfadiazine or sulfafurazole for about six weeks and in certain cases, for two-week periods each month thereafter for a long time.

Haschek in Vienna (6) has also recently emphasized the advantages of long-term therapy He used tetracycline combined with sulfamethoxypyridazine in high doses during the first three or four days, followed by a lower dosage of both drugs for several months. Of 40 patients, six became free from bacteriuria during the period of observation. Twenty-nine patients showed "clinical cures" five were asymptomatic during the treatment but developed subjective symptoms and cloudy urine when therapy was interrupted. On occasion sulfonamides instead of nitrofurantoin were given with good effect.

Hohenfellner in 1959 (8) confirmed the salutary effect of this therapeutic schema in 32 patients.

Johnson and Marshall in 1959 (11) treated chronic urinary infections prophylactically with nitrofurantoin for up to three years and followed up the patients for one to five years. The dosage was 1—5 mg/kg of body weight daily No serious toxic reaction occurred in their series. Urinary tract infection was successfully controlled with nitrofurantoin in 78 of 83 patients under observation for at least one year

Long term treatment of chronic pyelonephritis has been carried out at this clinic for approximately three years. In some cases the therapeutic regimen employed has been similar to those previously described but for the most part the schedule of treatment has differed to some extent Hence, a report of our therapeutic results may be of interest. In most of our cases, the diagnosis was verified by kidney biopsy and the kidney function was studied by *inter alia* estimation of the renal extraction of para-aminohippuric acid (PAH)

Table II. Clinical and laboratory data of chronic pyelonephritis prior to long-term treatment of 36 female patients

	No. of cases	% of determinations	Not determined
Anemia (< 11 g Hb/100 ml blood)	19	53	—
Low alkali reserve in blood (< 50 vol% CO_2)	6	17	—
Serum creatinine (> 2 mg % of whole blood)	13	36	—
Positive C-reactive protein	17	53	4
Bacteriuria (> 100,000 bacteria/ml)	34	94	—
Pathologic urinary sediment (numerous leukocytes and renal epithelial cells)	34	94	—
Concentrating power (< 450 m. osmol./l)	21	64	3
Diluting power (> 200 m. osmol./l)	6	17	—
Ammonium chloride load (pH of urine > 5.0)	33	92	—
PAH extraction (< 85 %)	24	86	8
Difference in PAH extraction between left and right kidney (> 10 %)	9	50	18
Difference in urine osmolarity between left and right kidney (> 25 m. osmol./l)	20	74	9
Pathologic roentgen findings	28	78	—
Arterial blood pressure exceeding 145/90 mm Hg	7	19	—
Hypertensive eyeground changes			
Moderate	9	27	3
Slight	5	15	
Renal biopsy indicating chronic pyelonephritis	20	80	11

Material

The series consists of 36 patients (all women) with chronic pyelonephritis who were given long-term treatment. They were received in the clinic during the period 1958—1960. The diagnosis was based upon the clinical history and routine examinations including urography cystoscopy and renal function tests. Urine cultures with bacterial count and determination of the bacterial sensitivity to antibiotics and chemotherapeutic drugs were performed in all cases. Each of the cases in this series manifested at least three of the following signs recurrent bouts of pyelonephritis or cystitis, bacterial count of more than 100,000/ml in morning specimen of urine substantial number of leukocytes and renal epithelial cells in the urinary sediment positive roentgenologic findings such as shrunken kidneys or abnormal calyceal contours and reduced concentrating power. In 28 of the patients the renal PAH extraction was studied by means of renal vein catheterization. In 20 cases the diagnosis was confirmed by renal biopsy.

All cases showing evidence of obstructive uropathy urolithiasis or other foreign bodies (such as sequestered papillae) or neurogenic diseases (such as multiple sclerosis, spinal cord lesions) were excluded.

Clinical and laboratory data recorded before treatment are listed in table II and the frequency of some symptoms in table I. The duration of symptoms is not included because of the long history and the insidious onset of the disease in most of the subjects. All the cases may be considered severe since they had been unsuccessfully treated in other hospitals and referred to this clinic for further therapy.

Table III Degree of sensitivity of one strain of E. coli at differing pH in urine

pH	Sulfamethyl thiadiazole mg/%	Nitrofurantoin mg/%	
6.4	20	1.5	Sensitive
7 0	> 200	12	Resistant
>7.8	> 200	> 12	Resistant

Table IV Incidence of pathogens in 36 females with chronic pyelonephritis prior to treatment

Type of infection	No. of cases
Coliform bacteria (E. coli, typical E. coli, Aerobacter aerogenes)	26
Proteus mirabilis and vulgaris	4
Coliform as well as Proteus bacteria	5
No bacteria	1

Method of treatment

The long term treatment was based on a chemotherapeutic drug, a sulfanilamide or nitrofurantoin. This treatment was initiated at the very outset and was continued throughout the entire period during which the patient was regularly examined, as to whether or not bacteriuria was present. In many cases this therapy sufficed to allay even the acute initial phase as well as acute relapses, and to depress or abolish the bacteriuria. In the majority of cases, however an adjuvant antibiotic was required during periods of acute symptoms (usually one to two weeks)

The choice of drug was based largely on determination of the bacterial sensitivity and the dosage on the functional condition of the kidneys. All patients with suspected chronic pyelonephritis were hospitalized for a thorough investigation which included bacterial cultivation with sensitivity tests, as well as kidney function determinations.

When the bacteria showed sensitivity to sulfanilamide or nitrofurantoin the relevant drug was used, but when th bacteria were resistant to both drugs *in vitro* sulfanilamide was given since it is well known that sulfanilamides are often effective *in vivo* against a strain of bacteria exhib ting *in vitro* resistance This difference in bacterial sensitivity may be attributable to differences between the pH of the culture media and that of the *in vivo* environment (tissues, urine) (17 19)

This pH dependence is illustrated by some experiments carried out by the author in collaboration with Dr Mai Arwidsson of th State Bacteriologic Laboratory A train of *Escherichia coli* was found to be resistant to both sulfamethizole and nitrofurantoin t a pH of 7 0 or higher whereas it was fairly sensitive to both drugs at a pH of 6.4. In case of infection with this strain the results of drug therapy were more favorable when the urine was kept acid by administration of ammonium chloride (table III) Incidence of pathogens is shown in table IV

The following antibiotics were used penicillin, streptomycin (21) chloramphenicol (23) tetracycline (5) kanamycin (4) and cycloserine (7) The choice depended on the result of the bacterial sensitivity tests.

The chemotherapeutic drug was chosen on the same basis. A sulfanilamide derivative was employed in the majority of cases, sulfamethizole being preferred in only a few exceptions. The dosage was 0.5—1 0 g three times or more daily depending on the renal function and the type and intensity of the infection. The plasma concentration of free sulfa drug was frequently checked with the aim of maintaining a concentration of 1 to 3 mg/100 ml blood No toxic reaction and no crystal deposits in the kidneys were observed. Nausea was very rare, occurring only when the dosage had become too high and the plasma drug concentration exceeded 6 to 7 mg/100 ml.

In long term treatment with sulfamethizole the rapid renal excretion of this drug however presents a difficult problem. Because of its high clearance (14) the tablets have to be administered several times a day in order to prevent the plasma drug concentration from falling below a therapeutically active level. If on th other hand, sulfanilamides with a lower clearance are used, the concentration in the urine becomes too low unless the dosage is so high as to give rise to nausea or toxic symptoms.

In order to obviate these difficulties a new tablet, Sulfapral® was evolved in co-opera-

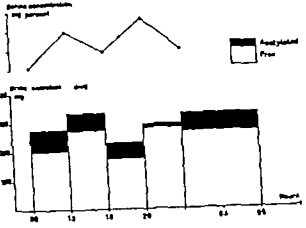


Fig. 1 36-year-old woman with chronic pyelonephritis. Concentration of sulfonamides in serum and urine after 12 days treatment with Sulfapral® (1 g × 2). End. creat. clear. 69 ml/min. Serum creatinine 1.1 mg %.

tion with the manufacturing druggists AB Astra (Södertälje, Sweden). This preparation consists of combination of sulfapyridazine and sulfamethizole. A part of the sulfamethizole is mixed with resin which retards its absorption in the intestine. The relevant dosage and the therapeutic results obtained with this preparation have been reported elsewhere (9 24). I most of the patients the suitable dosage was one to two tablets twice daily. The plasma drug concentration, which was frequently checked, was maintained between 5 and 10 mg/100 ml. A typical case is illustrated in fig. 1.

Nitrofurantoin was given (principally in cases infected with *E. coli*, *Aerobacter aerogenes* and *Proteus*) in dose of 50 mg three times daily for dosage of 150 mg per day to patients with normal serum creatinine level (below 2 mg/100 ml). Patients with creatinine concentration of 2 to 4 and in excess of 4 mg/100 ml received, respectively daily dosage of 100 mg and 50 mg nitrofurantoin. Due to technical difficulties the plasma concentration of nitrofurantoin was not determined, nor was urine concentration, except in few cases.

Some of the patients treated with nitrofurantoin complained of malaise, but side-effects and toxic symptoms were otherwise rare. I three cases neurologic signs such as slight paresthesia and numbness of the hands and feet were noted. The possibility that these symptoms were due to the renal insufficiency cannot, however be ruled out.

Results

The series has been divided into two groups on the basis of the long-term therapeutic results.

In the first group (32 cases) it was possible to control the infection, i.e. to eliminate or to suppress it. In 17 of these patients this was achieved by long-term therapy with a sulfanilamide and for nitrofurantoin, singly or in conjunction (administered either simultaneously or at different periods). In the other 15 it was necessary to supplement the chemotherapy with high doses of antibiotics for one or more short periods. Twenty-six of th patients had *Coliform* bacteria, four had *Proteus* bacteria, and one had both. In one patient who had a typical history of pyelonephritis as well as characteristic symptoms, bacteriuria of unknown origin had earlier been detected at another hospital. In each of these patients the subjective symptoms were more or less completely eliminated. Slight and vague symptoms such as occasional headache or backache persisted in a few instances. As a rule, all clinical symptoms including the urinary sediment disappeared. These

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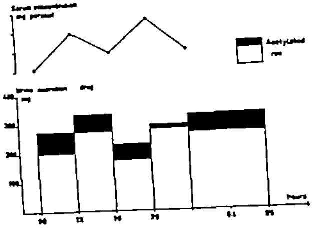


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Results

The series has been divided into two groups on the basis of the long-term therapeutic results.

In the first group (32 cases) it was possible to control the infection, i. e. to eliminate or to suppress it. In 17 of these patients this was achieved by long term therapy with a sulfanilamide and for nitrofurantoin, singly or in conjunction (administered either simultaneously or at different periods). In the other 15 it was necessary to supplement the chemotherapy with high doses of antibiotics for one or more short periods. Twenty-six of the patients had *Coliform* bacteria, four had *Proteus* bacteria, and one had both. In one patient who had a typical history of pyelonephritis as well as characteristic symptoms, bacteriuria of unknown origin had earlier been detected at another hospital. In each of these patients the subjective symptoms were more or less completely eliminated. Slight and vague symptoms such as occasional headache or backache persisted in a few instances. As a rule all clinical symptoms including the urinary sediment disappeared. These

Table III Degree of sensitivity of one strain of E. coli at differing pH in urine

pH	Sulfamethyl-thiadiazole	Nitro-furantoin	
	mg/%	mg/%	
6.4	20	1.5	Sensitive
7 0	> 200	12	Resistant
>7.8	> 200	> 12	Resistant

Table IV Incidence of pathogens in 36 females with chronic pyelonephritis prior to treatment

Type of infection	No. of cases
Coliform bacteria (E. coli, atypical E. coli, Aerobacter aerogenes)	26
Proteus mirabilis and vulgaris	4
Coliform as well as Proteus bacteria	5
No bacteria	1

Method of treatment

The long term treatment was based on a chemotherapeutic drug a sulfanilamide or nitrofurantoin. This treatment was initiated at the very outset and was continued through out the entire period during which the patient was regularly examined, as to whether or not bacteriuria was present. In many cases this therapy sufficed to allay even the acute initial phase as well as acute relapses, and to depress or abolish the bacteriuria. In the majority of cases, however an adjuvant antibiotic was required during periods of acute symptoms (usually one to two weeks)

The choice of drug was based largely on determination of the bacterial sensitivity and the dosage on the functional condition of the kidneys. All patients with suspected chronic pyelonephritis were hospitalized for a thorough investigation which included bacterial culti vation with sensitivity tests, as well as kidney function determinations.

When the bacteria showed sensitivity to sulfanilamide or nitrofurantoin the relevant drug was used, but when the bacteria were resistant to both drugs *in vitro*, sulfanilamide was given since it is well known that sulfanil-amides are often effective *in vivo* against a strain of bacteria exhibiting *in vitro* resistance This difference in bacterial sensitivity may be attributable to differences between the pH of the culture media and that of the *in vivo* environment (tissues, urine) (17 19)

This pH dependence is illustrated by some experiments carried out by the author in collaboration with Dr Mai Arwidsson of the State Bacteriologic Laboratory A strain of *Escherichia coli* was found to be resistant to both sulfamethizole and nitrofurantoin at a pH of 7 0 or higher whereas it was fairly sensitive to both drugs at a pH of 6.4. In cases of infection with this strain the results of drug therapy were more favorable when the urine was kept acid by administration of ammonium chloride (table III) Incidence of pathogens is shown in table IV

The following antibiotics were used penicillin, streptomycin (21) chloramphenicol (23) tetracycline (5) kanamycin (4) and cycloserine (7) The choice depended on the result of the bacterial sensitivity tests.

The chemotherapeutic drug was chosen on the same basis. A sulfanilamide derivative was employed in the majority of cases, sulfamethizole being preferred in only a few exceptions. The dosage was 0.5—1 0 g three times or more daily depending on the renal function and the type and intensity of the infection The plasma concentration of free sulfa drug was frequently checked with the aim of maintaining a concentration of 1 to 3 mg/100 ml blood No toxic reaction and no crystal deposits in the kidneys were observed. Nausea was very rare, occurring only when the dosage had become too high and the plasma drug concentration exceeded 6 to 7 mg/100 ml.

In long term treatment with sulfamethizole the rapid renal excretion of this drug however presents a difficult problem. Because of its high clearance (14) the tablets have to be administered several times a day in order to prevent the plasma drug concentration from falling below a therapeutically active level. If on the other hand, sulfanilamides with a lower clearance are used the concentration in the urine becomes too low unless the dosage is so high as to give rise to nausea or toxic symptoms.

In order to obviate these difficulties a new tablet, Sulfapral® was evolved in co-opera

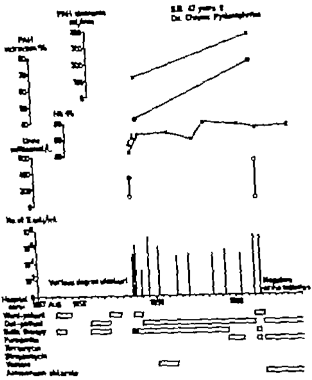


Fig. 2. 47-year-old woman with chronic pyelonephritis.

Seven patients were subjected to autovaccination against *E. coli* without positive results.

Discussion

The frequent relapses and reinfections make the therapy of chronic pyelonephritis very difficult task. The infection frequently recurs when therapy is interrupted even though the urine has long been free from bacteria and all other symptoms have disappeared. According to Kass (12) not more than 10 of the patients with chronic pyelonephritis are permanently cured.

Reinfections occurred in 14 of the 36 cases in this series (39 %). Some of these had several relapses of bacteriuria caused by different strains of bacteria, indicating reinfection via a *locus minoris resistentiae* as above. The relapses were detected early and the patients subjected to intensified therapy. Thus 86 % of the cases were free from relapses for one year or more during long-term therapy. An illustrative case is the following:

A 47-year-old nurse was examined at the clinic in September 1958 after having had several attacks of pyelonephritis since 1957. Continuous treatment with sulfanilamide (sulfamethizole for more than one year) and with streptomycin for short periods had succeeded only in depressing the coliuria (initial count approximately 16 mill/ml). When the bacteria were found to be resistant to sulfonamide, nitrofurantoin (50 mg × 3) was given for about three months, but the bacteriuria still persisted. She was again hospitalized in April 1960 and for 11 days received streptomycin, supplemented for the first five days by tetracycline. Under this treatment the urine

Table V Results of long-term treatment in 36 females with chronic pyelonephritis

Duration of therapy	Type of therapy				
	S	S + A	N + A	S + N	N+S+A
Infection eliminated or suppressed					
3 —6 months	–	2	–	2	–
>6 —12 months	4	2	1	2	–
>1 —1½ years	4	2	–	–	1
>1½—2 years	3	1	–	–	2
>2 —2½ years	2	–	–	–	3
>2½—3 years	–	–	–	–	1
Persistent infection					
6 —12 months	–	–	–	–	1
>1 —1½ years	–	–	–	–	1
>1½—2 years	–	–	–	–	1
>2 —2½ years	–	–	–	–	1
Total no. of pat.	13	7	1	4	11

S = sulfonamides. N = nitrofurantoin. A = antibiotics.

Table VI Prolonged therapy with sulfamethylthiadiazole for E. coli bacteriuria in 12 females with chronic pyelonephritis

	1–7 days	8–14 days	15–30 days	>1–6 months	>6–12 months	>1–1½ years	>1½–2 years	>2½ years
Treatment duration until first negative culture (number of patients)	3	2	2	2	1	–	–	–
Duration of long-term therapy	–	–	–	–	4	3	3	2

Mean no. of bacteria/ml before treatment	< 100 000	100,000–1 mill.	1,1–10 mill.	10,1–100 mill.	100,1–200 mill.
Number of patients	–	1	2	8	1

patients have been treated for periods varying between three months and three years. Except in a few cases chemotherapy is still proceeding (table V)

The other group comprises four cases in which bacteriuria persisted despite intensive treatment with both chemotherapeutic agents and antibiotics. The clinical and subjective symptoms were nevertheless, either alleviated or abolished Both *Coliform* and *Proteus* bacteria were present in the urine of all four These patients had been treated for one to three years.

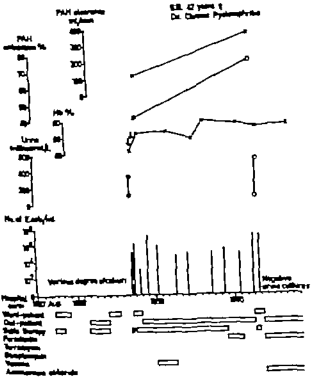


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Table V Results of long-term treatment in 96 females with chronic pyelonephritis

Duration of therapy	Type of therapy				
	S	S + A	N + A	S + N	N + S + A
Infection eliminated or suppressed					
3 —6 months	–	2	–	2	–
>6 —12 months	4	2	1	2	–
>1 —1½ years	4	2	–	–	1
>1½—2 years	3	1	–	–	2
>2 —2½ years	2	–	–	–	3
>2½—3 years	–	–	–	–	1
Persistent infection					
6 —12 months	–	–	–	–	1
>1 —1½ years	–	–	–	–	1
>1½—2 years	–	–	–	–	1
>2 —2½ years	–	–	–	–	1
Total no. of pat.	13	7	1	4	11

S = sulfonamides. N = nitrofurantoin. A = antibiotics.

Table VI Prolonged therapy with sulfamethylthiadiazole for E. coli bacteriuria in 12 females with chronic pyelonephritis

	1–7 days	8–14 days	15–30 days	>1–6 months	>6–12 months	>1–1½ years	>1½–2 years	> 2½ years
Treatment duration until first negative culture (number of patients)	5	2	2	2	1	–	–	–
Duration of long term therapy	–	–	–	–	4	3	3	2

Mean no. of bacteria/ml before treatment	< 100,000	100,000–1 mill.	1.1–10 mill.	10.1–100 mill.	100.1–200 mill.
Number of patients	–	1	2	8	1

patients have been treated for periods varying between three months and three years. Except in a few cases chemotherapy is still proceeding (table V).

The other group comprises four cases in which bacteriuria persisted despite intensive treatment with both chemotherapeutic agents and antibiotics. The clinical and subjective symptoms were nevertheless either alleviated or abolished. Both *Coliform* and *Proteus* bacteria were present in the urine of all four. These patients had been treated for one to three years.

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became, and has since remained abacterial. No relapse has occurred during subsequent prophylactic treatment with nitrofurantoin and ammonium chloride. Her renal function had already shown a considerable improvement before the urine became sterile, i. e. during the period, when the bacteria were depressed by therapy. The concentrating and diluting power had increased and the renal extraction of PAH was two times the original value, moreover the hemoglobin concentration had spontaneously risen from 6.4 to 11.1 g/100 ml. Two minor exacerbations of the pyelonephritis occurred during the period of bacteriuria but they were readily suppressed by intensified nitrofurantoin therapy. Aside from occasional headache and backache the patient has no subjective symptoms (fig. 2).

At this clinic our therapeutic experience of chronic urinary tract infections, especially pyelonephritis, has led us to favour long term chemotherapy with adjuvant antibiotics during acute phases or attacks. A reduction of the coagulase and toxin contents of the blood may be expected. Frequent and thorough examinations are essential, however, for detection of every acute relapse as early as possible. With this procedure it should be possible to prevent destruction of the renal parenchyma and to maintain the patient in good condition.

Summary

The superiority of continuous long term antibacterial therapy of chronic pyelonephritis is emphasized. This form of therapy was employed in 36 cases treated at the Renal Clinic during the period 1958—1960. *Coliform* bacteria were found in the urine of most of these patients.

The therapy consists of continuous administration of a chemotherapeutic drug supported by antibiotics during acute bouts or relapses. In most of these cases sulfamethizole was employed as the chemotherapeutic agent but in a few cases nitrofurantoin was the drug of choice.

On completion of the treatment period reported here, we switched over to use a preparation containing sulfamethizole and sulfamethoxypyridazine when sulfonamide therapy was preferred (9, 24).

In 32 of the 36 long term therapy cases the infection was eliminated or considerably depressed and the frequency of relapses decreased. Most of the patients (30 cases) remain under treatment. The duration of treatment was between three months and three years.

On elimination or amelioration of the bacteriuria the renal function improved. It is assumed that with this treatment the danger of kidney parenchyma destruction and hence of relapses and reinfections will be lessened.

Acknowledgement

The bacteriologic work was done at the State Bacteriologic Laboratory by Dr. Birgit Backelin, to whom we are greatly indebted for valuable and stimulating discussions.

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Low Subvalvular Aortic and Pulmonic Stenosis Caused by Asymmetrical Hypertrophy and Derangement of Muscle Bundles of the Ventricular Wall

by

S. Bevegård, B. Jonsson and I. Karlöf

Isolated infundibular stenosis of the right ventricle is characterized by hypertrophy and dislocation of the two muscular bands, the septal and the parietal, going out from the crista supraventricularis (Kjellberg et al. 1959). This causes a localized stenosis in the ostium infundibuli. The stenosis can be more pronounced depending on fibrous endocardial thickening. The subvalvular aortic stenosis as a rule is caused by a fibrous ring situated immediately below the valves. In some cases, a muscular band, from which the fibrous tissue originates, can also take part in the formation of the constriction (Gruenwald 1947, Greenberg and Simon 1949, Brachfeld and Gorlin 1959).

Recently Goodwin et al. (1960) have described another type of subvalvular aortic stenosis, caused by a localized hypertrophy of the ventricular septum. The pathological anatomy had previously been described by Teare (1958) as "asymmetrical hypertrophy of the heart". The microscopical picture is characterized by "a coarse texture with bizarre arrangements of bundles of muscle fibres separated by connective tissue and clefts". The localization of this tumor-like hypertrophy determines the type of the functional disturbance. In one of Teare's cases, where the process was situated in the anterior wall of the left ventricle, the mitral orifice was constricted. Hollman et al. (1960) have described cases with obstruction of the tricuspid orifice. The most common localization, however, seems to be in the ventricular septum.

It is possible that other types of processes might result in the same functional disturbance. In a general survey in 1925, Uehlinger reported 7 cases of solitary rhabdomyoma, and since then further cases of solitary tumor in the myocardium have been described (Bradley and Maxwell 1928, Browne and Gray 1930). It is not possible to judge if these cases also revealed signs of stenosis.

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Left ventricular impulse	Auscultation	Heart vol. ml	Total hemoglobin g	Blood vol. l	Work kpm/min. at pulse rate 170/min.
Heaving	Normal sounds. Systol. murmur grade 4 over 4th LIS. Faint mid-diastolic murmur over apex	1,650	640	4.8	700
Heaving	Second sound faint. Systol. murmur grade 5 over apex and along left sternal border up to 2nd LIS. Short faint, late diastol. murmur 2nd—4th LIS	1,220	543	3.8	400
Normal	Normal sounds. Mid-systol. murmur grade 3 over apex. Faint, mid-diastolic murmur over apex	770	—	—	650
Heaving	Normal sounds. Systol. murmur grade 4. Faint, mid-diastol. murmur over apex	1 130	774	6.2	1,000
Heaving	Normal sounds. Systol. murmur grade 4 over apex	1,240	745	5.7	600
Heaving	Normal sounds. Mid-systol. murmur grade 4 over apex	1,280	—	—	—
Normal	Split second sound. Systol. murmur grade 4 over 2nd LIS. Faint mid-diast. murmur over 4th LIS	1 045	560	4.5	300
Heaving	Normal sounds. Systol. murmur grade 4, over apex	970	733	5.3	500

ever the description is very incomplete regarding clinical symptoms and autopsy findings and makes it impossible to draw conclusions about the kind of functional disturbance.

During 1959—1961 we have studied 8 cases which seem to be identical with those described by Goodwin et al. (1960). In all cases there was a stenosis within the left ventricle and two of them also revealed one in the right ventricle. All cases have been examined with heart catheterization. The result of the roentgenological studies including cardioangiography has been reported by Nordenström and Ovenfors (1962).

Methods

Electrocardiogram at rest and during work was recorded with the technique described by Holmgren et al. (1959). *Phonocardiogram* was recorded with an Elema ink jet-recorder. *Physical working capacity* (PWC_{170}) defined as the working intensity in kpm/min. performed at a pulse rate of 170 beats per minute, was determined on an electrically braked bicycle ergometer with a work load increasing stepwise every 6 minutes (cf. Sjöstrand 1960). Using the approximately linear relationship between pulse rate and work load, the value of the physical working capacity is obtained by extra- or intrapolation. In this material the pulse rate on the highest load varied between 143 and 169 beats per minute. *Total amount of hemoglobin* (THb) was determined with the alveolar CO-method (Sjöstrand 1948) and the *blood volume* was calculated from the

Table I

Case no.	Sex	Age years	Height cm	Weight kg	B. S. A. m	Symptoms
1	F	32	165	62.5	1,68	Since early childhood dyspnea increasing at age 18. Since one year fatigue, palpitation but no precordial pain
2	F	35	155	60 0	1.56	Since early childhood dyspnea increasing the last years. Since two years palpitation and precordial pain
3	M	37	162	64 0	1 68	Since age 29 palpitation and precordial pain
4	M	38	174	69.5	1.84	Since age 31 dyspnea palpitation and precordial pain
5	M	42	169	67.5	1 78	Since age 15 dyspnea palpitation and precordial pain increasing the last 3 years
6	M	43	178	79.0	1 98	Active in sport to age 36. Since age 37 precordial pain and dyspnea
7	F	41	162	63.0	1.66	Since childhood precordial pain. Since few years fatigue and dyspnea
8	M	38	164	62.0	1.68	Since age 36 feeling of substernal oppression on exertion. No typical angina pectoris

During recent years several cases have been described in which a pressure gradient between the left ventricle and the aorta was found but where no valvular stenosis or subvalvular fibrous ring was observed during operation (Brachfeld and Gorlin 1959 Brent et al 1959 Brock 1959 Morrow and Braunwald 1959 Soulié et al 1959 Liveeay et al 1960) In some of these cases the upper part of the ventricular septum was bulging into the left ventricle below the aortic valves. This disease may be the same as that described by Goodwin et al (1960) Cases reported by Davies (1952) and Neufeld et al. (1960) probably also belong to this group A series of 14 cases, including the cases earlier reported by Morrow and Braunwald (1959) has been described by Braunwald et al. (1960)

As a rule the stenosis affects the left ventricle Neufeld et al (1960) however report two cases in which the outflow region of the right as well as of the left ventricle was constricted One of the cases was a stillborn infant and the other died at the age of one month No hemodynamic investigations were performed. In 1910 Bernheim described cases with left ventricular hypertrophy where a bulging septum was supposed to cause stenosis within the right ventricle How

Left ventricular impulse	Auscultation	Heart vol. ml	Total hemo-globin g	Blood vol. l	Work kpm/min. at pulse rate 170/min.
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THb and the hemoglobin concentration of finger blood. *Heart volume* was determined in the prone position with exposure during quiet breathing (Larsson and Kjellberg 1948) The procedures and methods for *right heart catheterization* as used in our laboratory have been described by Bevegård et al. (1960) The patients were studied in supine position. Cardiac output was determined according to the direct Fick method with analysis of blood gases spectrophotometrically and of expired air according to Haldane and Priestly (1935) In case 6 the right heart catheterization was made sometime earlier at another hospital and is not reported here.

For *left heart catheterization* different methods were used. In cases 2, 5 and 6 the pressure was measured after direct puncture of the left ventricle, immediately before injection of contrast medium. Simultaneously the pressure was measured in the ascending aorta through a catheter introduced from the brachial artery according to Seldinger's technique (1953) These examinations were made a few days after right heart catheterization and under general anesthesia. The difference between the two examinations concerning pulse rate and systemic arterial pressure is seen in table II Cases 1 7 and 8 were examined under local anesthesia, and left ventricular pressure was obtained through a catheter passed in a retrograde direction from the aorta. The pressures in the left ventricle and aorta were measured from a withdrawal curve, while the ECG showed regular sinus rhythm. In case 4 left heart catheterization was performed under local anesthesia, using a transseptal puncture technique (Bevegård et al. 1961) In case 3 no left heart catheterization was performed

Results

The main symptoms, physical findings and some anthropometric data are presented in table I The age varied between 32 and 43 years. Four cases had symptoms since childhood and four cases developed symptoms later in life. The oldest patient was an active athlete until the age of 36 and he had symptoms only during the last 5 years. The symptoms were dyspnea, palpitation and precordial pain None had any history of rheumatic fever or cardiac decompensation. Cases 1 and 7 had however earlier been treated with digitalis.

Cases 1 and 4 belonged to a family with 10 children among whom none of the others had known heart disease. Cases 2 and 7 belonged to families where many deaths from cardiac disease had occurred. The available information, however did not permit exact diagnosis concerning the heart disease.

At *physical examination* increased left ventricular impulse was found in all cases except for nos 3 and 7 The heart sounds were normal in all cases except in no 7 where the second sound was abnormally split. The predominant auscultatory finding was a rather loud systolic murmur with its maximal intensity over the apex or the left sternal border in the 4th costal interspace. In case 2 where in addition a stenosis within the right ventricle was found the murmur was loud also in the second left interspace. In case 7 the murmur had its maximal intensity in the second left intercostal space and was very faint over the apex. The murmurs were of high frequency in all cases and started late in systole, as a rule about 0.10 sec. after the first sound (fig 1) Five cases also had a diastolic murmur which in four cases was situated in mid-diastole and in one (case 2) during the atrial systole

The *electrocardiogram* was abnormal in all cases (fig 2) There were marked ST and T changes over the left ventricle in all cases except no 3 and 7 In case 3 the ST-segments and T waves were depressed only in leads II III and aVF Case 7 showed a quite different ECG pattern with depressed ST-segments in lead CR_1

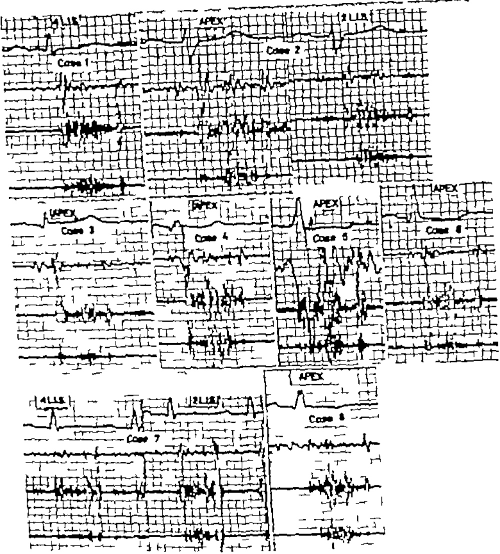
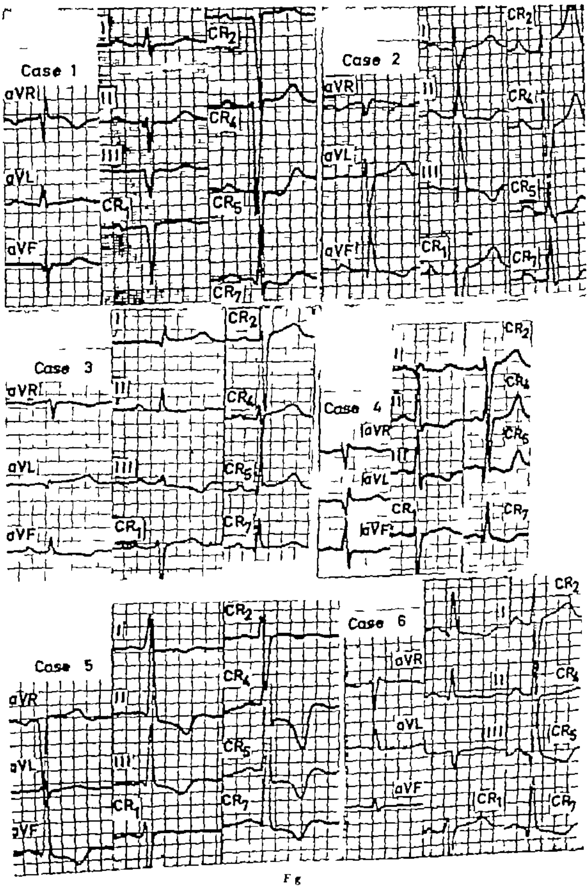


Fig. 1 Phonocardiogram (see text)

CR and CR The R/S-ratio was high in lead CR_1 and CR and low in CR_6 and CR The P-waves were abnormally high in cases 2 and 7 In several cases the ECG signs of left ventricular hypertrophy were more pronounced than could be expected with regard to the degree of hemodynamic disturbance (cf fig 2 and table II) Cases 4 and 6 had small pressure gradients but showed as marked ECG changes as case 1 who had a pronounced pressure gradient.

It is remarkable that case 2, having the predominant hemodynamic changes within the right ventricle, did not show ECG signs of right ventricular hypertrophy but only changes corresponding to the left ventricle.


Case 1
aVR
aVL
aVF
I
II
III
CR1
CR2
CR4
CR5
CR7
Case 2
Case 3
Case 4
Case 5
Case 6

F g

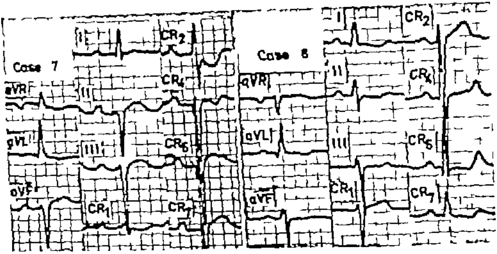


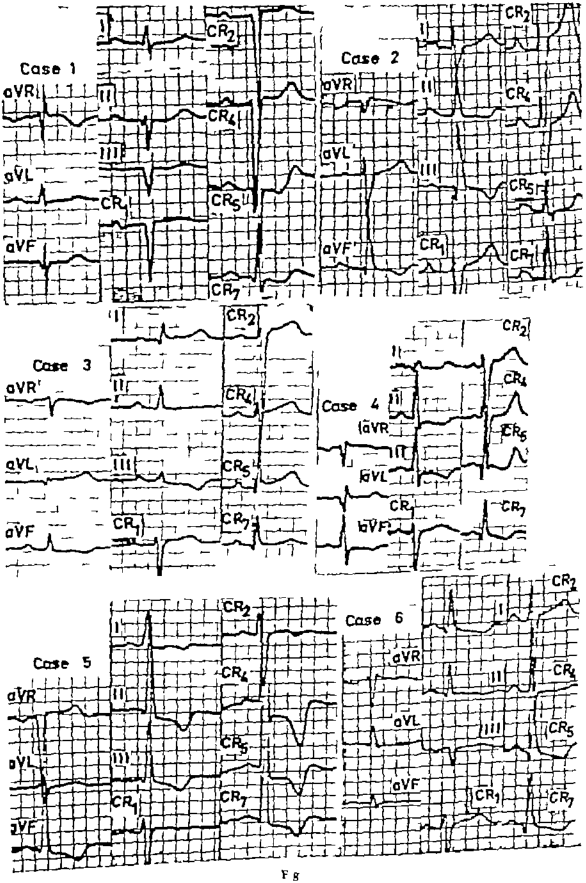
Fig. 2. Electrocardiogram (see text)

The heart volume was large in all cases except no. 5. The most marked cardiac enlargement was found in case 1. A more exact evaluation of the degree of cardiac enlargement will be obtained if the heart volume is related to the total amount of hemoglobin (fig. 3) and the deviation from the normal regression line is determined (Holmgren et al. 1957). In a similar way the *physical working capacity* is estimated in relation to the total amount of hemoglobin (fig. 4). With cardiac disease where the heart volume increases and the physical working capacity decreases, the deviation from the normal variation will be most pronounced if the physical working capacity is related to the heart volume (Kjellberg et al. 1949) (fig. 5). All cases, except no. 5 showed considerable deviation from the normal variation. As case 6 was not investigated with regard to the functions mentioned above it is difficult to estimate to what extent the disease had caused pathological increase in heart volume. The patient's heart was large (1,280 ml) but he had earlier been an active athlete, and as shown by Holmgren and Strandell (1959) the heart volume in athletes remains large even a long time after cessation of physical training.

During the estimation of the physical working capacity only one case (no. 5) got precordial pain so severe that the work had to be stopped at a fairly low pulse rate (146 beats per min.). Case 4 felt only a slight pressure over the precordial region when working up to a pulse frequency of 162 beats per min. The other 4 cases, investigated with the exercise test, had no pains although the work was continued up to pulse frequencies varying between 143 and 169 beats per min. According to the history none of these patients had typical angina on effort.

Heart catheterization

For thorough evaluation of the degree of stenosis the pressure gradient and the stroke volume should be determined simultaneously and during a circulatory steady state. These requirements have been fulfilled only in cases 4, 7 and 8. Puncture of the left ventricular wall and


Case 1
aVR
aVL
aVF
I
II
III
CR1
CR2
CR4
CR5
CR7
Case 2
Case 3
Case 4
Case 5
Case 6

Fig

Pressures, mm Hg														
RA	RV		PA			PCV	LA	LV				Aorta		
								Apex		Outflow				
M	S	De	S	D	M	M	M	S	De	S	De	S	D	M
3	33	6	33	17	23	18	—	—	—	—	—	123	68	86
—	—	—	43	25	30	23	—	—	—	—	—	150	80	94
—	46	9	46	27	33	26	—	—	—	—	—	150	80	100
—	—	—	—	—	—	—	—	192	11	107	35	111	61	78
0	114	6	26	7	13	8	—	—	—	—	—	121	67	84
—	—	—	—	—	—	—	—	128	11	—	—	81	45	—
3	42	5	32	9	17	11	—	—	—	—	—	118	60	84
—	96	7	77	37	58	37	—	—	—	—	—	164	69	97
4	21	5	21	6	13	—	7	—	—	—	—	101	56	71
—	—	—	—	—	—	—	—	131	25	111	25	111	66	—
1	25	0	25	5	13	—	—	—	—	—	—	109	59	76
—	—	—	—	—	—	—	—	—	—	111	12	116	60	88
—	—	—	—	—	—	—	—	125	19	—	—	96	63	—
4	Inflow 80 Outflow 53	3 6	53	27	38	—	—	229	29	122	26	122	78	—
1	35	2	28	9	16	11	—	153	14	96	16	87	60	68

RA = Right atrium RV = Right ventricle PA = Pulmonary artery PCV = Pulmonary capillary venous LA = Left atrium LV = Left ventricle M = Mean; S = Systolic, De = End-diastolic D = Diastolic.

also in the outflow tract. The pressure in the pulmonary artery at rest, was increased only in cases 1 and 7. The increased pulmonary arterial pressures were explained by the high diastolic pressures in the left ventricle.

Considerable stenosis in the outflow tract of the right ventricle was present in case 2. Case 7 had a slight stenosis. Pulmonary stenosis had been excluded in case 6 at the previously performed right heart catheterization. The slight pressure gradient of 10 mm Hg in case 3 increasing to 19 mm Hg during exercise, is not significant for stenosis. The considerable rise of pressure in the pulmonary artery and pulmonary veins during exercise in this case is pathological in relation to the age (Holmgren et al. 1960, Granath et al. 1961). The end-diastolic pressure of the right ventricle was slightly increased only in cases 1 and 2. Only in case 2 was the a wave in the right atrium abnormally high (12 mm Hg). The cardiac output in relation to oxygen uptake (fig. 6) was low only in cases 2 and 7 who also disclosed the lowest physical working capacities. There were no signs of any shunt.

Table II Data obtained in connection with heart catheterization

Case no.	Work load kpm/min.	Technique	Pulse rate beats/min.	Oxygen uptake ml/min.	O_2 capacity ml/100 ml	O_2-sat. per cent		AV-O_2-diff. ml/l	Card. output l/min.	Stroke volume ml
						Br A.	PA			
1	Rest	R. H. C.	101	315	17.5	100	72	51	6.2	60
	100	R. H. C.	128	664	18.1	97	54	80	8.5	63
	200	R. H. C.	152	887	18.3	97	49	89	10.0	76
	Rest	R. L. H. C.	102	—	—	—	—	—	—	—
2	Rest	R. H. C.	78	226	18.3	98	73	48	4.7	61
	Rest	L. V. P.	49	—	—	—	—	—	—	—
3	Rest	R. H. C.	86	231	17.4	94	78	32	7.[illegible]	84
	400	R. H. C.	150	1,3[illegible]7	20.4	96	45	133	12.5	84
4	Rest	R. H. C.	66	274	18.2	95	72	43	6.4	97
	Rest	T. S. C.	63	280	17.6	98	74	45	6.[illegible]	99
5	Rest	R. H. C.	62	303	18.3	94	70	45	6.7	108
	Rest	L. V. P.	83	—	—	—	—	—	—	—
6	Rest	L. V. P.	67	—	—	—	—	—	—	—
7	Rest	R. L. H. C. + R. H. C.	96	235	18.5	93	64	54	4.7	49
8	Rest	R. L. H. C. + R. H. C.	86	243	18.5	97	77	38	6.3	74

R. H. C. = Right heart catheterization R. L. H. C. = Retrograde left heart catheterization
L. V. P. = Left ventricular puncture T. S. C. = Transseptal catheterization Br. A. = Brachial artery PA = Pulmonary artery

general anesthesia can change the hemodynamics considerably. Pressure gradients measured during such conditions may consequently not be representative of basal conditions. In case 2 both the pulse rate and the systemic arterial pressure had changed markedly compared with the values found at right heart catheterization. In case 5 however the deviation was smaller. In our experience the best methods for hemodynamic studies of left sided heart lesions are the transseptal approach and the retrograde catheterization technique. As illustrated by case 4 a catheter passed through the mitral orifice to the left ventricle does not necessarily change pulse rate, pressure and stroke volume. The pressure gradient between the aorta and the apical region of the left ventricle was measured in 6 cases. In three of these cases the pressure gradient exceeded 50 mm Hg. The pressure in the apical region as well as in the outflow tract was measured in 4 cases (nos. 1, 4, 7, 8).

The end-diastolic pressure in the left ventricle was increased in cases 1, 4, 6, 7 and 8. The diastolic pressure was high

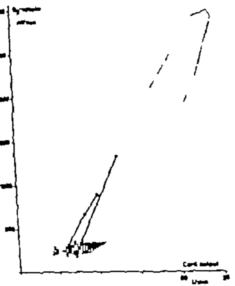


Fig. 6. Oxygen uptake in relation to cardiac output. The area of normal variation obtained from determinations on 28 healthy subjects (Bevegård et al. 1960, Holmgren et al. 1960) is delineated by dotted line. The hatched area represents the normal variation at rest.



Fig. 7. Cardioangiography in case 1. The injection of contrast medium was made into the left ventricle after transthoracic puncture. See Nordenstrom and Ovenfors (1962)

Nordenstrom and Ovenfors. They stated

In all cases the ventricular wall was considerably thickened and irregular indentations in the lumen of the left ventricle could be seen. The thickening, which was most marked at the borderline between the conus and sinus part of the ventricle 4—5 cm below the aortic cusps, caused the lumen of the left ventricle to appear divided into two parts. This was most prominent during ventricular systole.

By the aid of cardioangiography with injection in the left ventricle, moderate mitral regurgitation was found in case 2 and a slight regurgitation in cases 1 and 6.

Cases 2 and 6 were operated. They died few days later. Microscopic examination of the resected heart muscle showed hypertrophy with interstitial fibrosis.

Discussion

The clinical picture of this heart disease is generally so characteristic that a diagnosis can be made by physical examination, electrocardiography and ordinary roentgen examination. In certain cases, however the physical findings and ECG are atypical as well as the roentgenological appearance. This seems to depend upon differences in localization and extension of the myocardial process. At catheterization of the right ventricle it is usually impossible to distinguish between a stenosis caused by the muscular disease and an infundibular stenosis of the usual type. As regards the left ventricle it is true that the stenosis is

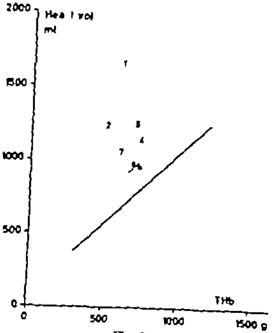


Fig 3

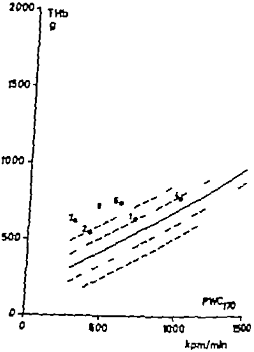


Fig 4

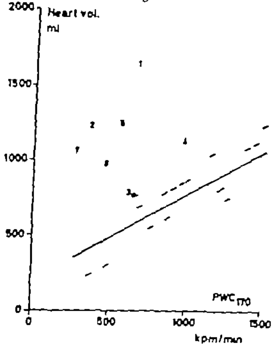


Fig 5

Fig 3 Heart volume in relation to total amount of hemoglobin (THb)

Fig 4 Total amount of hemoglobin (THb) in relation to working capacity (PWC_{170})

Fig 5 Heart volume in relation to working capacity (PWC_{170})

The straight lines in fig 3, 4 and 5 represent the normal regression lines obtained from determinations on 58 healthy subjects. Interrupted lines represent ± one and two standard errors of estimat (Holmgren et al. 1957) In case 3 the value for the heart volume in erect position is plotted since it was not determined in the prone position.

Roentgen examination

The findings on plain films and on cardioangiography in all 8 cases have been reported by Nordenström and Ovenfors (1962) The configuration of the heart was abnormal with enlargement of the left ventricle in all cases and enlargement of the left atrium in all cases except no 3 In five cases there was right ventricular enlargement and in four of them also enlargement of the right atrium The aorta was not dilated

The appearance of the malformed left ventricle is demonstrated in fig 7 which is a cardioangiogram from the paper by

situated more apically than the common type of subaortic stenosis. It is, however difficult to localize the site of the stenosis with certainty during catheterization. The method of choice for anatomical diagnosis is cardioangiography

By analyses of the sequence of films obtained at cardioangiography Nordenström and Ovenfors could demonstrate an abnormal contraction of the left ventricle. Between the conus and the sinus part of the left ventricle the trabeculi were arranged in a direction perpendicular to that usually seen in a normal left ventricle. This seems to explain the marked increase of the stenosis during systole dividing the left ventricle into two parts. The stenosis is therefore not so evident in an atonic heart as during an operation with extracorporeal circulation or at post mortem examination. Pressure recordings from the left ventricle proximal and distal to the stenosis also indicate the increase of the stenosis in late systole (fig 8)

These anatomical and hemodynamic features explain the physical findings. Thus the murmur is heard and recorded late in systole is maximally heard over the apex or at left sternal border in the 4th left interspace and unlike the murmur of valvular aortic stenosis is not transmitted to the neck vessels. In case 7 the murmur was maximal at the 2nd left interspace probably due to the stenosis of the right ventricular outflow tract. In spite of the marked stenosis near the apex of the left ventricle no murmur was heard or recorded over that area even with phonocardiographic recording from the esophagus.

Four cases showed faint mid-diastolic murmurs over the apex. They may be explained by whirl formation in the left ventricle during the phase of rapid filling due to the distortion of the ventricular cavity. Mitral stenosis could be excluded in case 4 where pressures were simultaneously recorded from the left atrium and left ventricle. In case 2 a diastolic murmur was heard during atrial systole, projected over the 2nd—4th left intercostal spaces. In this case the filling of the right ventricle was probably accomplished to a great extent during atrial systole. In the pressure recording from the right ventricle (fig 8) the transmitted atrial wave is clearly seen and it is considerably higher in the sinus part of the ventricle than in the outflow tract. The diastolic murmur can be explained by the flow through the ventricular stenosis during the heavy atrial systole.

In many cases with a rather mild stenosis there were marked electrocardiographic signs of left ventricular hypertrophy The ECG changes are therefore probably to a great extent caused by the primary myocardial process and not only by a hypertrophy secondary to the stenosis. Even case 2 with marked stenosis of the right ventricle but only a slight stenosis of the left, had ECG changes typical of left ventricular hypertrophy At the same time there were P waves as seen in right ventricular hypertrophy Case 7 who had a marked pressure gradient in the left ventricle and an insignificant gradient in the right ventricle had, unlike the other cases, no definite signs in the ECG of left ventricular hypertrophy (fig 2) Pressure recordings from the right ventricle showed a delay of the isometric contraction of 0,05 sec. in comparison with the left ventricle. Cardioangiography showed a markedly thickened septum, which might explain the delay of activation of the right ventricle This fact also explains the marked splitting of the second sound.

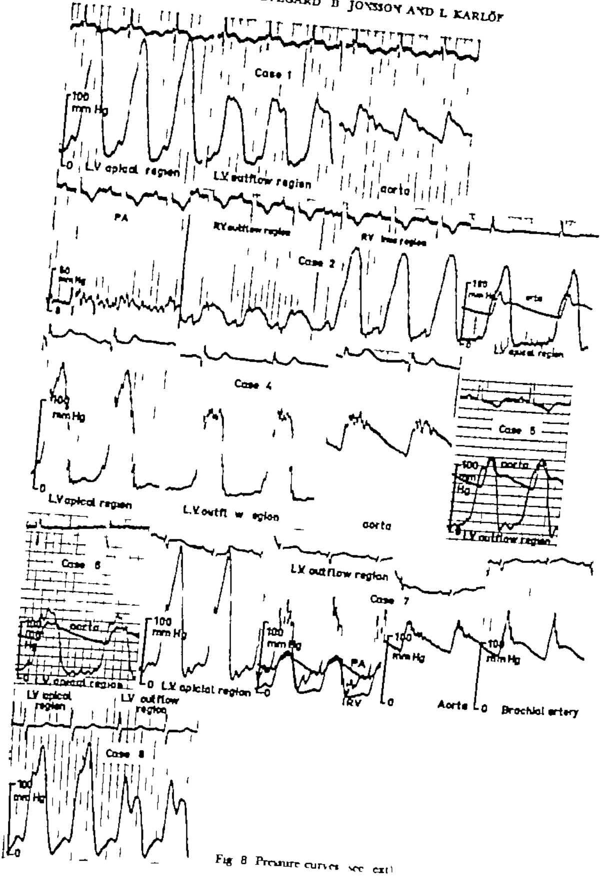


Fig. 8. Pressure curves, see text.

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The low stroke volume in relation to the heart volume may be an expression of impaired myocardial function. This was most clearly demonstrated in cases 1 2 and 7 In case 2 the discrepancy between the effective stroke volume and the heart volume may partly be explained by a moderate degree of mitral regurgitation In the remaining cases it is an expression of increased residual blood volume of the heart These cases had marked changes also in the right ventricle and they also had the most marked hemodynamic changes with low cardiac output (fig 6)

The pressure curves registered from the aorta (fig 8) had not a slow uprise at the start of systole as is usually seen in valvular or high subvalvular aortic stenosis. Good win et al (1960) made the s me observation in their cases.

This disease tends to be hereditary (Davies 1952 Teare 1958, Brent et al 1959 Braunwald et al 1960 Hollman et al 1960) Two of our cases were brother and sister and two other cases belonged to families where several members had died of heart disease of unknown cause It is most probable that the disease is not uncommon since several cases from different parts of the world have been published during the last few years

Summary

Eight cases with low subvalvular aortic stenosis have been described Two of them had also a narrowing with a pressure gradient in the right ventricle The stenosis is caused by an asymmetrical hypertrophy and an abnormal course of the muscle bundles of the ventricles with a disturbance in the mode of contraction. The stenosis is more marked during late systole as seen from cardioangiography and pressure recordings. The murmur is therefore late systolic and its maximal intensity is heard over the apex or at left sternal border at the 4th left interspace. The electrocardiogram shows signs of left ventricular hypertrophy which are more marked than expected from the degree of stenosis.

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A Case of Hyperthyroidism Developed in Spite of Previous Hypophysectomy

By

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The normal function of the thyroid gland is dependent upon the pituitary production of thyroid stimulating hormone (TSH). Following hypophysectomy in man (Li et al. 1955) clinical signs of myxoedema will usually develop in 6 to 8 weeks. This is in agreement with results from animal experiments, and autonomous regulation of thyroid function has consequently been considered of minor importance (Tamrog et al. 1958). We have recently had the opportunity to see a patient who became thyrotoxic one year after an apparently complete hypophysectomy. A similar clinical paradox has been observed in a few other instances (Fajans 1958, Gurling et al. 1959, Werner & Stewart 1958).

Case report

A woman, aged 71, had been in perfectly good health until 1952, when she noted a lump in the right breast. A radical mastectomy was performed and she had no complaints for the following 6 years. In 1958 she developed back pains and an X-ray gave evidence of metastases in the eighth, ninth and tenth thoracic vertebral bodies. Her general condition was good, and she was advised to undergo hypophysectomy which was performed by the transsphenoidal route in Oct. 1958.

Prior to this operation no clinical signs of hyperthyroidism were noted. A BMR was + 16, the pulse rate was around 70, but PBI had not been done.

Her back pains disappeared soon after the hypophysectomy and she was discharged with a daily dose of 7.5 mg prednisolone and 60 mg desiccated thyroid.

She was first seen by us in Nov. 1960 when she came to the out-patient clinic for routine investigation of her thyroid function. On that occasion she complained of being a little nervous and of sweating and palpitations. She was without pain or any other complaint. Her appearance gave slight suspicion of thyrotoxicosis. She had no goitre and no adenoma. The PBI was found to be a little on the high side, namely 9.1 μg/100 ml. A radioiodine thyroid uptake study gave a 24 hours uptake of 63 % and PBI (24 hrs after the dose) 0.12 % of dose per litre plasma.

Considering the previous hypophysectomy and the fact that she was on thyroid medication, this was certainly a surprising finding. The desiccated thyroid was stopped. An I uptake study 5 weeks later gave, however, essentially the same result.

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are in agreement with the clinical impression of the thyrotoxicosis being rather mild.

Of particular interest seems the fact that the 24 hour I^{131} uptake increased after administration of triiodothyronine. This result has been found in several of our thyrotoxic patients and was first observed by Shimizu et al. (1960).

The explanation of this phenomenon is not clear but the present observation of its occurrence in a patient lacking the pituitary gland may be of interest. A possible interpretation may be, that triiodothyronine has a direct effect on the thyroid, inhibiting the release of its hormones to some extent, whereas the radioiodine uptake is unaffected by the administration of T_3.

The high I^{131} uptake may possibly be caused by an abnormal thyroid activator which is operating in thyrotoxicosis, and which not is influenced by exogenous triiodothyronine.

No pituitary gonadotropin could be detected in the urine, and its content of the androsterone fraction of the 17 ketosteroids was very low. These results suggest that the hypophysectomy was complete. Indirect evidence for this may also be found in the fact, that by microscopy of serial cuts no residual pituitary tissue has been found in those cases, who later came to autopsy and who had been operated on by the same surgeons.

Persistent thyroid function was found by Pazianos et al. (1960) in 10 patients out of a series of more than 350 cases of hypophysectomy performed in patients with metastatic mammary carcinoma. In 4 of these there was evidence of functioning adenomas of the thyroid. Albeaux Fernet et al. (1955) described the effect of hypophysectomy in a patient with hyperthyroidism and severe exophthalmos. Becker (1959) reported on another two similar cases. In these patients the toxic state was ameliorated by the hypophysectomy which did, however not result in any cessation of thyroid function.

The paradox of thyrotoxicosis developing in patients with little or no functioning pituitary tissue has been observed in some single other cases. Fajans (1958) has reported on a patient who became thyrotoxic after having suffered from postpartum necrosis of the pituitary for twenty years. In this patient the thyroid gland showed advanced atrophy with only small local areas of hyperplasia. A thyrotoxic patient observed by Werner and Stewart (1958) had a chromophobe pituitary adenoma leaving only a very small remnant of normal pituitary tissue. Gurling et al. (1959) have described two patients in whom thyroid adenomas developed many months after they had a hypophysectomy performed. One of these patients became thyrotoxic.

The fact that adenomas may function without stimulation by their trophic hormone is not unique for the thyroid gland. Autonomous adrenocortical tumours may be mentioned in this connection.

In the present case there were no palpable thyroid nodules. The existence of a retrosternal adenoma was, however not ruled out and nor could the presence of a number of small adenomatous areas within the gland be excluded.

The works of McKenzie (1958) Adams (1958) and Munro (1959) have demonstrated an abnormal thyroid stimulator in the serum of some thyrotoxic patients. This agent L.A.T.S. ("long-acting thyroid stimulator") accelerates thyroid secretion in assay animals for a longer period than does T.S.H. The latter can be separated from L.A.T.S. by extraction by an alcohol percolation procedure. It is

Her condition was followed at intervals and there were no clearcut clinical signs of hyperthyroidism

From April 1961 however she began loosing weight, and she was admitted to the hospital In the period from April 1961 to Aug 1961 the following laboratory investigations were performed

Radioiodine thyroid uptake (May 8)

4 hour uptake 62 (normal 15—45)
24 " 66 (" 30—70)
PBI ¹³¹ 0.2 of dose per litre serum (24 hrs after dose)

Radioiodine thyroid uptake (May 15)

(after suppression with triiodothyronine (120 μg daily for 6 days))
4 hour uptake 64
24 " " 81

Radioiodine thyroid uptake (Aug 28)

4 hour uptake 98.5
PBI ¹³¹ 0.22 of dose per litre serum (24 hrs after dose)
Equipment for doing a thyroid scanning was not available.
PBI (each value represents the mean of 3 determinations)
11.6 μg/% (July 18) 10.8 μg/ (July 19) 11 μg/ (Aug 31)

Erythrocyte uptake of triiodothyronine
15.3 (July 18) 17 3 (Aug 29) (normal range 7—10.5)
BMR + 21 (June 26) + 15 (June 27) — 9 (Sept. 4)
Weight 49.5 kg (April 21) 47 kg (June 7) 45 kg (July 7)
Pulse rate around 90 for most of this period
Serum cholesterol 225 mg (June 19) 205 mg % (Aug 28)
Serum phosphor 2 3 mg alkaline phosphatases 7 2 King Armstrong U (June 14)
BSR gave several times a value of around 15 mm/hour
Serum transaminase 26 U (normal values below 40 U)
X ray of spinal column showed no increase in metastatic destruction. No other metastases were detected
To evaluate the completeness of the hypophysectomy the excretion of pituitary gonadotropins was determined on Nov 24 1960, and on April 29 1961 On both occasions no gonadotropins could be detected (the 24 hour excretion being less than 3 mouse U). Before the hypophysectomy was performed, she excreted 64 U./24 hour (normal range 17—150 mouse U (Johnsen 1958))

A determination of th different fractions of 17 ketosteroids (April 24) showed a very low excretion of the androsterone fraction (0.02 mg/24 hours) (normal range 0.6—3.1).

By the end of the observation period mentioned above she was mildly thyrotoxic. Thyroid tissue could be felt, but there was no goitre and no adenoma. Since the above laboratory investigations clearly indicated a hyperthyroid state she was started on neomercazole 15 mg per day

She was seen again 2 months later and her condition had definitely improved. She felt no nervousness and had no palpitations. She had gained 7 kg in weight, and the PBI had come down to 7.2 μg

Radioiodine thyroid uptake (Nov 13 1961)

4 hour uptake 34
24 " 32
PBI ¹³¹ 0.07 of dose per litre plasma
Erythrocyte uptake of triiodothyronine 11.8 (normal range 7—10.5)

Discussion

It seems evident from the laboratory data cited that the patient was suffering from thyrotoxicosis. The high PBI values and the increased erythrocyte T_3 uptake values are in favour of this diagnosis. So are the high thyroid I ¹³¹ uptake values, which were also found while the patient was on thyroid medication. The administration of triiodothyronine did not result in any suppression of thyroid uptake of radioiodine. On the contrary a small increase in the 24 hour uptake was found. The PBI¹ value (0.21) was above the average for normal persons. In most of our thyrotoxic cases, however values higher than 0 4 of dose per litre serum was found The comparatively low PBI values and the moderately increased BMR

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Hirudoid — Anticoagulant Ointment

A Failure to Demonstrate Significant Effect

By

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Hirudoid ointment contains a heparinoid substance prepared from animal organs, presumably in such a form that it can be absorbed percutaneously. Numerous favourable reports are available. According to the manufacturer and these publications, Hirudoid ointment has an antithrombin activity (9, 16), is a fibrinolytic agent (16), induces hyperaemia and has an antiphlogistic effect. Based on these effects a long list of indications has been suggested. It has been used in the treatment of thromboembolic diseases, in the complications of varicose veins, in paronychia, furunculosis, tendovaginitis, bursitis, haematoma, pleurisy and to prevent postoperative thromboembolic diseases (2, 3, 4, 5, 9, 10, 11, 12, 13, 15, 17, 19, 22). Because of the anticoagulant activity the main indication is thromboembolic disease.

The ointment is placed on the skin and the dosage is determined by measuring the length of the ointment string from the tube. According to the manufacturer 6 g daily divided into two doses, is sufficient. With this dose one gets maximum effect, about 40—50 % prolongation of the whole blood clotting time, and greater effect has not been obtained by increasing the dose.

The present work was started as a result of an application to market the drug in Norway. The anticoagulant effect of the ointment was studied in normal persons. In no case could a satisfactory effect be demonstrated, and the preparation is therefore considered to be unreliable and therefore potentially dangerous. These negative results are reported because they are in conflict with all the previous favourable reports.

Material

Heparin AL. (identical to heparin "Vitrum", Stockholm, Sweden) containing 50 mg or 5,000 I. U. per ml was diluted in saline to the desired strength.

Hirudoid extract, the active material of the ointment, was dissolved in saline to the desired strength.

Hirudoid ointment (Luitpold-Werk, Munich, Germany) contains Hirudoid extract 10, glycerin 14, thymol 0.17, massa unguentum ad 100. The ointment was kindly supplied by the producer.

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not known where this abnormal thyroid activator is produced The present case might possibly find its explanation in a thyroid stimulation by such agent This would evidently exclude the pituitary gland as the production site of the "long acting thyroid stimulator

Summary

A case of hyperthyroidism developed one year after a complete hypophysec tomy is reported The contribution of this observation to the understanding of the regulation of thyroid function is discussed

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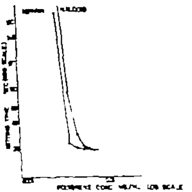


Fig. 3. The neutralization of heparin and Hirudoid-extract with increasing amounts of polybrene.

Test system: 0.2 ml platelet-poor citrated human plasma containing heparin or Hirudoid-extract and 0.2 ml polybrene solution of various strengths were incubated at 37° C for 3 min. 0.2 ml of the thrombin solution was then added. The thrombin solution was diluted to give thrombin time in normal plasma of 20 secs. The plasma contained 0.01 mg/ml of Hirudoid-extract or 0.005 mg of heparin.

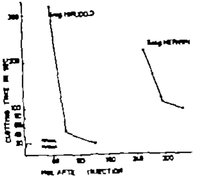


Fig. 4. The effect of intravenous injection of 5 mg Hirudoid and 5 mg heparin.

Test system: 0.2 ml platelet-poor citrated rabbit plasma and 0.2 ml saline were incubated at 37° C for 3 min. 0.2 ml thrombin solution was then added (5 I. U. per ml).

active principle in the Hirudoid ointment, was first tested and compared to the anticoagulant effect of heparin.

Anticoagulant effect. Increasing amounts of Hirudoid and heparin were added to platelet poor citrated plasma, and the antithrombin effect was measured in the thrombin system. Fig. 1 shows that increasing amounts of Hirudoid extract and heparin prolonged the thrombin time. The curves for Hirudoid extract and heparin have the same shape, but heparin has stronger antithrombin activity than Hirudoid extract on weight basis.

For the in vivo experiments, it was important to know the sensitivity of the thrombin system to the two anticoagulants. As shown in fig. 1 the curve is horizontal for Hirudoid concentrations below 0.0001 mg/ml. This is therefore the smallest amount of Hirudoid extract that can be detected in this system. A more sensitive system was obtained by reducing the strength of the thrombin solution (see fig. 2). About 0.00003 mg/ml could then be detected. However as stated previously the experimental error was greater when the thrombin time was increased.

Neutralization of Hirudoid with polybrene. Polybrene interacts stoichiometrically with heparin, and 0.9 mg polybrene neutralizes 1 mg heparin (8). Fig. 3 shows the effect of various amounts of polybrene on heparin and Hirudoid plasma. With increasing amounts of polybrene, the thrombin time became shorter and the curves changed from vertical to horizontal: at this point, the neutralization point, the heparin or Hirudoid was neutralized. On the basis of the neutralization point, the concentration of heparin or Hirudoid in the plasma can be calculated. The curves have the same shape, but a greater amount of polybrene was needed to neutralize 1 mg of heparin than 1 mg Hirudoid extract. However, in terms of anticoagulant effect polybrene neutralized more heparin than Hirudoid, because heparin had greater anticoagulant activity. The curves show that 1 mg of polybrene neutralizes about 1.1 mg of heparin and about 1.7 mg of Hirudoid extract.

In vivo experiment in rabbits. The effect of heparin disappears rather quickly in vivo, and injections every 4—6 hours are required to maintain therapeutic effect. For the following work it is of interest to know the disappearance rate of Hirudoid extract after intravenous injection. Hirudoid extract is not available prepared for intravenous injection in man; the following experiments were there

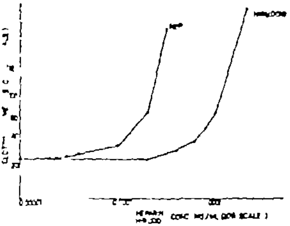


Fig. 1. The effect of various amounts of heparin and Hirudoid-extract on the thrombin time of platelet-poor citrated human plasma.

Test system: 0.2 ml platelet poor citrated human plasma and 0.. ml heparin or Hirudoid extract solution of various strengths were incubated at 37° C for 3 min. 0.2 ml of the thrombin solution was then added. The thrombin solution was diluted to give a thrombin time in normal plasma of 25 secs.

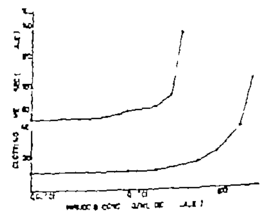


Fig. 2. The sensitivity of the thrombin system to Hirudoid-extract at two different strengths of the thrombin solution.

Test system: 0.2 ml platelet-poor citrated human plasma and 0.2 ml Hirudoid-extract sol tions of various strengths were incubated at 37° C for 3 min. 0.2 ml of the thrombin solution was then added. In the upper curve the thrombin solution was diluted to give a thrombin time of 30 secs. with normal plasma, and in the lower curve to a thrombin time of 14.8 secs. with the same plasma.

Human citrated platelet-poor plasma. Nine parts of whole blood were collected through a siliconized needle into one part of a 3.1 sodium citrate dihydrate solution in a lusteroid tube. The mixture was centrifuged for 30 min at 2,500 r. p. m. (1 400 g) and the platelet poor plasma was pipetted off and stored in lusteroid tubes at + 4 C.

Polybrene (poly 1 5-dimethyl-1 5-diazaundecamethylene methobromide) from Abbott Laboratories, North Chicago, Ill U.S.A. was diluted in saline to the desired strength.

Test persons were healthy men without any known blood diseases or serum protein changes which could interfere with the thrombin time.

Thrombin. A bovine thrombin Topostasine (Hoffmann La Roche, Basel, Switzerland) was dissolved in saline to the desired strength.

Rabbit citrated plasma. Two ml whole blood were collected from a cut in the ear vein into 0.4 ml 3.1 % sodium citrate dihydrate solution in a siliconized glass tube. The mixture was centrifuged for 30 min at 2,500 r. p. m. (1 400 g) and the plasma was pipetted off and stored in siliconized glass tubes at + 4 C.

Methods

Thrombin time. 0.2 ml platelet-poor citrated plasma and 0.2 ml saline were incubated in a glass tube at 37° C for 3 min. then 0.2 ml of thrombin was added and the time necessary for the formation of a solid clot was recorded. The mean of 4—6 parallel tests was taken as the thrombin time. With short thrombin times, this system has a low experimental error and gives reproducible results. With very dilute thrombin solutions, the experimental errors become greater because it is more difficult to determine the end point accurately. The thrombin time was determined in plasma as soon as possible after the blood had been collected and centrifuged.

Whole blood clotting time. Three clean, new glass tubes were filled to the 1 ml mark directly by venepuncture with a siliconized needle and immediately placed in a water bath at + 37° C. All glass tubes were gently tilted every 30 sec. The mean clotting time of three tubes was recorded.

In vitro experiments. The anticoagulant effect of the Hirudoid extract, which is the

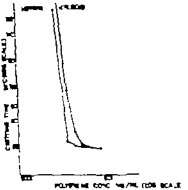


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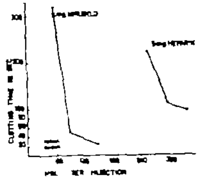


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Table I. The thrombin time before and after application of 10 g Hirudoid ointment on the back and on the forearm. For the first 4 persons a short thrombin time was used, for the others a more sensitive system with a longer thrombin time was used

Test person no.	Thrombin time before ointment application (secs.)	Thrombin time after ointment application on the back (secs.)		
		3 hrs	6 hrs	8 hrs
1	12.6	12.8	13.4	13.2
2	10.7	10.9	11.2	11.9
3	12.8	11 4	12.5	11 4
4	12.2	11.9	12.5	11 7
5	28.2	27.2	27.6	28.0
6	4 4	24 1	25.0	24.5
7	31.5	31 6	32.2	32.6
8	48.1	62.0	48.4	50.2
9	44.9	44..	45.3	44.2
10	44.6	44 6	42.0	42.1
11	45.6	46.3	44.5	43.8
		Thrombin time after ointment application on the forearm blood collected from the same arm (secs.)		
		1 hr	2 hrs	3 hrs
12	51.3	50.5	5..0	50.1
13	47 6	47 6	48.8	47.2
14	47.5	46.8	49 6	47 5
15	51.8	49.6	49.5	52.0

Test system 0.2 ml platelet-poor citrated human plasma and 0.. ml saline were incubated at 37 C for 3 min. 0.2 ml thrombin solution was then added.

fore performed in rabbits. Five mg of Hirudoid extract was dissolved in saline and injected intravenously into rabbits. In parallel experiments, the same amount of heparin was injected. The effect was measured with the thrombin time.

Fig. 4 shows that the effect of Hirudoid disappeared much faster than the effect of heparin under these experimental conditions.

Table II. The whole-blood clotting time before and after application of 10 g Hirudoid ointment on the back and on the forearm

Test person no.	Whole-blood clotting time before ointment application (secs.)	Whole-blood clotting time after ointment application on the back (secs.)		
		3 hrs	6 hrs	8 hrs
1	203	221	205	208
2	210	240	250	260
3	180	210	200	247
4	270	240	223	220
5	281	300	300	322
6	230	265	215	240
7	230	227	224	273
		Whole-blood clotting time after ointment application on the forearm blood collected from the same arm (secs.)		
		1 hr	2 hrs	3 hrs
1	270	55	240	284
13	210	240	250	220
14	40	250	250	250
15	240	210	255	225

Test-system Three clean new glass tubes were filled to a 1 ml mark directly by venepuncture with a siliconized needle and immediately placed in a water bath at 37 C. All glass tubes were gently tilted every 30 sec. The mean of the three clotting times is recorded.

However in terms of *effect* (not in mg) the dose of heparin was much higher. The only conclusion that can be drawn is therefore that the effect of Hirudoid does not disappear more slowly than that of heparin.

In vivo experiments in man. The in vitro experiments showed that Hirudoid extract had a significant antithrombin activity. To study the effect of the ointment in man, the whole blood clotting time and the thrombin time were measured before and after application of 10 g ointment on the skin of healthy persons.

In order to compare the thrombin time before ointment application, and after 3—6 and 8 hours, the activity of the trombin solution must be kept stable. The thrombin solution was stored in lusteroid tubes at + 4° C. This method was found to preserve unchanged activity for at least 12 hours.

Table I shows the thrombin time before and after the application of 10 g Hirudoid ointment in an area measuring 20 × 35 cm on the back. Since the thrombin solution was stable during each experiment, the thrombin times before and after the application of ointment may be compared. For the first four test persons a relatively short thrombin time was used, and no significant effect could be demonstrated. The test system was therefore made more sensitive by increasing the thrombin time. However no effect could be demonstrated.

I the next experiment the ointment was applied to the forearm, and the blood was collected from vein draining the area of ointment application. Ten g Hirudoid ointment as used. However even in this experiment, no effect of the ointment could be demonstrated (see table I).

Table II shows the whole-blood clotting time after application of Hirudoid ointment on the back and on the forearm, as above. As is seen from the tables, no significant prolongation of the clotting time could be demonstrated.

Discussion

The in vitro experiments show that Hirudoid extract has a significant antithrombin effect, but this effect is considerably less than that of heparin, on a weight basis. Hirudoid was also neutralized by polybrene but the potency of polybrene against Hirudoid was greater than that against heparin, on a weight basis. When injected intravenously into rabbits, the effect of Hirudoid extract disappeared t least as rapidly as that of heparin.

In all these experiments, Hirudoid extract acted as a heparinoid. Many investigators have postulated (1 2 3 9 16 21) that Hirudoid ointment has an anticoagulant effect in vivo. They have found that the effect becomes measurable some 2 hours after application, maximum effect is attained after 4—6 hours, and there is a demonstrable effect for 24 hours after application of 6 g ointment. According to the same investigators, the maximum effect — 40-50 % prolongation of the whole-blood clotting time — cannot be further increased by using more than 6 g Therefore, they claim that there is no danger in using the ointment, and no blood control is necessary during treatment. This maximum effect of a limited amount of ointment is hard to explain, if we accept that the ointment has any effect in vivo at all. Shedel (20) has tried to obtain a greater effect by increasing the dose, but did not succeed in this. This contrasts with the in vitro results in the present work (fig 1 and 2)

There is no doubt that Hirudoid extract contains an anticoagulant principle, but I could not demonstrate any antithrombin or anticoagulant effect in man after application of 10 g ointment. This is 4 g more than the manufacturer recommends as the maximum dose. In a double blind test Eriksson (6) did not find any difference between the placebo and the Hirudoid group in preventing thrombophlebitis after intravenous injections. The ineffectiveness of the ointment in this experiment can easily be explained in the light of my observations. The percutaneous absorption is slow and a small dose of Hirudoid disappears quickly if and when absorbed. Since the absorption is too slow and the elimination is too fast, the concentration of Hirudoid in the blood does not become great enough to give a significant anticoagulant effect.

The sensitivity of the thrombin system, as used in this work, is shown in fig 1 and

2. This test system cannot be compared with those used by other investigators, because the sensitivity of their systems to Hirudoid extract has not been stated However the sensitivity of the system now used is great enough to demonstrate antithrombin activity of therapeutic value which is the essential point.

As mentioned above a long list of indications for Hirudoid has been suggested but it is hard to explain all the effects which the ointment is supposed to have

There is some disagreement among the various investigators about the indications for Hirudoid treatment. Some use it in superficial thrombophlebitis only in more extensive processes the commonly used anticoagulants are recommended The latter authors thereby admit that the common anticoagulants are more effective than Hirudoid in the treatment of thromboembolic disease. The only reason for using Hirudoid ointment treatment is that this entails no risk of bleeding complications. Hirudoid treatment thus means an undertreatment, and this is generally more dangerous than overtreatment. With the simple control methods available today the risk of bleeding complications during anticoagulant treatment is far less than the risk of complications by undertreatment of thromboembolic disease

Shedel and Daniel (20 3) recommend Hirudoid in thromboembolic prophylaxis after surgery In these patients the effect of the ointment cannot be explained by the local and superficial activity

Finally in discussing the indications for Hirudoid treatment, no one has discussed whether the stated 40—50 % prolongation of the whole-blood clotting time is enough to be therapeutically effective in thromboembolic disease The requirements for prolongation of the whole blood clotting time vary but 40—50 % is less than that commonly recommended.

Conclusion

In the treatment and prophylaxis of thromboembolic disease, the Hirudoid ointment cannot be recommended. It is potentially dangerous, since it may be used in patients whose lives can only be saved by *effective* anticoagulant therapy

Summary

In the in vitro experiments Hirudoid extract has an antithrombin activity which could be neutralized by polybrene. No antithrombin activity could be demonstrated in the blood from healthy test persons after application of 10 g Hirudoid ointment.

The ointment is not recommended in the treatment of thromboembolic disease.

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Summary

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Acute Pericarditis Associated with Coxsackie B-5 Virus Infections

By

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During the early winter of 1960—1961 several patients suffering from lymphocytic meningitis were admitted to the epidemiology department of Oslo Municipal Hospital. During the same time many cases with symptoms of fever, pleurodynia, and headache were reported from the city. On a number of hospitalized patients Coxsackie B-5 virus was isolated from faecal or spinal fluid specimens received in the virus laboratory. Few enteroviruses of other types were isolated during the same time. Two adult patients had symptoms of acute pericarditis with laboratory evidence of Coxsackie B-5 virus infections. The case reports are given together with the virological examinations. The observations furnish further evidence that these viruses may not infrequently be responsible for this syndrome.

Case reports

Case 1. A woman 52 years old, was admitted to the hospital Nov. 1 1960 with fever 38.9° C and pleurodynia. The illness had started 8 days previously with sudden attacks of pain in the epigastrium and later thoracic pains. The pain was accentuated by breathing. She was ordered to bed by her doctor and in the course of two days her temperature returned to normal, although her pain remained. The day after admission a pericardial friction rub was heard which lasted two days. In the ECG (4 standard leads) the T waves became isoelectric and later inverted. After 6 weeks the ECG was again normal. X-ray the day after admission showed pericardial and bilateral pleural effusion. A new X-ray two weeks later was normal. The pains gradually disappeared, and she was discharged Dec. 1 completely recovered.

Virological examinations. From faecal specimens taken Nov. 2 and 5 no virus was isolated. A blood specimen taken on the day of admission (8th day of illness) showed neutralizing antibodies against Coxsackie B-5 virus in a dilution of 1/512 and a second specimen taken 3 months later showed a titre of 1/64.

Case 2. A man 21 years old, was admitted Jan. 3, 1961 with clinical signs of pericarditis. The illness had started 10 days previously with attacks of thoracic pain which were accentuated by breathing. The pain disappeared after 2 days, he felt well, and returned to work. The day before admission the pain reappeared without rise in temperature. In hospital a typical pericardial friction rub was heard over the precordium, which persisted for two weeks. Apart from the pain the patient felt well during the first four days in hospital.

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He then suddenly felt very ill and presented signs of vascular collapse: cyanosis, tachycardia, and drop in blood pressure. This episode lasted one day, whereafter he gradually improved. ECG was normal on the day of admission, but on the following day the T waves in the four standard leads were iso-electric, and became inverted on the second day. On day of discharge the ECG still showed T wave changes although with notable regression. Thoracic X-ray on 5th, 11th and 17th Jan. did not reveal anything abnormal.

Virological examinations. Coxsackie B-5 virus was isolated from a faecal specimen taken on the first day in hospital. A second isolation trial 14 days later was negative. A blood specimen from the day of admission presented neutralizing antibodies against Coxsackie B-5 virus in a dilution of 1/512, and a second specimen taken three weeks later had a titre of 1/1024.

Discussion

The virological findings in these two cases of clinically benign acute pericarditis are sufficient evidence of deeper infections with the Coxsackie B-5 virus. In the first case no virus was isolated, but a decrease in neutralizing antibodies in the blood serum during the three months following the illness is considered good laboratory evidence in favour of the diagnosis. In the second case the virus was isolated at the time of the disease from the faeces of the patient, and high neutralizing antibodies were demonstrated in the serum. In both cases the illness presented a diphasic course and had started 8 to 10 days before the first blood specimen was taken. This is sufficient time for the neutralizing antibodies to develop in high titres, and a diagnostic rise in titre during the acute phase could not be demonstrated in any case. Virus and antibodies may be demonstrated as signs of infection, which however may coincide with a disease of other aetiology, but the occurrence of the pericarditis during an epidemic of Coxsackie B-5 infections favours the notion that the virus was responsible. The crucial proof would be the isolation of the agent from the pericardial effusion, but this has, to our knowledge, never been done.

Cardiac complications of infections by Coxsackie and possibly other enteroviruses in older children and adults have not been reported frequently but may have been overlooked (1). Known clinical manifestations of Coxsackie B virus infections are (6):

1. Aseptic meningitis. 2. Pleurodynia. 3. Undifferentiated illness with pharyngitis. 4. Myocarditis or encephalomyocarditis during early childhood and neonatal period. 5. Mild paralysis or encephalitis.

Fatal myocarditis in the newborn has been described which was caused by representatives of this group of viruses (3, 4). During the last few years reports have appeared that acute benign pericarditis in adults may be caused by representatives of this group of viruses. Fletcher & Brennan (2) have reported a case with pericarditis associated with Coxsackie B-4 virus in a 25-year-old man. Weinstein (9) has reported pericarditis associated with a post illness decrease in neutralizing antibody titre against Coxsackie B-5. Movitt et al. (7) have reported two cases of pericarditis associated with Coxsackie viruses A 1 and B-3. The patients were 22 and 74 years old respectively, and in both cases the virus agent was isolated and a rise in neutralizing antibodies during the acute phase of illness demonstrated. This suggests that also other enteroviruses than the Coxsackie B group may be responsible. Lately Lewes and Lane (5) have reported a case of be-

nign acute pericarditis, associated with Coxsackie B-3 virus in a 32-year-old man. Virus isolation from faeces was performed and neutralizing antibody titre rise was demonstrated.

It seems therefore, that acute benign pericarditis in the adult should be added to the list of clinical manifestations of Coxsackie B virus infections.

The correlation of many enterovirus infections with clinical syndromes is still a matter of research. Many viruses have been isolated from faeces of healthy and diseased humans but it is yet unknown whether they may provoke disease (10).

The laboratory diagnosis is based either on the isolation of the virus or the demonstration of a titre in neutralizing antibodies in serum during the disease.

Preferably the virus should be isolated from the site of infection (pericardial effusion). At an early stage of disease, the virus is present in the faeces. The amount of virus in the faeces seems to decrease quite rapidly and this explains the frequent failure in isolation trials on patients admitted to hospital when the disease has lasted for some time. The same reservations apply to the demonstration of a neutralizing antibody titre rise which usually occurs during the first 10 days of infection.

This rise is ascertained by titration of two serum specimens taken 7—10 days apart (acute phase, and convalescent phase serum). The demonstration of large amounts of neutralizing antibodies against a virus type in a single specimen is not considered enough evidence for the laboratory diagnosis, but when the disease occurs during an epidemic with the same virus, the titre may be quite suggestive.

Retrospectively a second blood specimen taken some months after the disease may by comparison with the specimen taken during the illness permit a demonstration of a decrease in antibody titre, which is also considered laboratory evidence for a diagnosis.

The epidemiological circumstances are of importance from both the diagnostic and public health points of view. It is a common experience that the enteroviruses may interfere with one another. The reported cases occurred during a period with many Coxsackie B-5 virus isolations on the patients from the Oslo area. During the preceding period the most common virus isolation in our laboratory was polio type I. After the Coxsackie B-5 type appeared during the autumn of 1960 type I polio was only isolated once in a group of persons making a family epidemic. A shift from polio type I to Coxsackie B-3 and B-5 was observed on isolations made in London about the same time (8).

In most cases the virus diagnosis will be established late in the patient's illness and is of little therapeutic importance. The aetiological diagnosis in this field is mostly of epidemiological and public health interest.

Summary

The authors report two cases of pericarditis in adults associated with Coxsackie B-5 virus infection.

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The Peripheral Blood Flow in Intermittent Claudication

I. The Significance of the Postural Tests

By

Leif K. Hillestad

In intermittent claudication of the calf the relevant subject for study is the blood flow through the calf muscles. This can be estimated by means of plethysmography. However plethysmography is technically difficult and not suitable for routine use.

The clinical examination of the ischemic limb must therefore still rest upon the old methods of pulse palpation, postural tests, skin thermometry, oscillometry and measurement of claudication-distance. By these methods the degree of impaired arterial blood flow is tentatively assessed. Consequently their value is dependent on the extent to which they reflect the actual blood supply to the limb.

In the present work the significance of the postural tests in intermittent claudication is evaluated by matching them with the calf blood flow as obtained by means of plethysmography. Similar studies of skin thermometry, oscillometry and claudication-distance will appear in later communications. Finally the plethysmographic studies will be described separately.

Intermittent claudication was first described in the horse by the veterinary surgeon Bouley (1831), later in man by Charcot (1858). It consists of pain in the calf provoked by walking and may occur in hypothyroma (7), diseases of joints and muscles, spinal cord lesions (8, 17), lumbar disc protrusions (2) and venous obstructions (15). However, the majority of cases are caused by obliterative arterial disease of varied etiology.

Material

The patients studied in this work, were women and men from 40 to 65 years of age. Their disease was intermittent claudication due to obliterative arteriosclerosis. None of them had detectable disease elsewhere, and none had noticeable trophic lesions of the feet.

The prevalent lesion was partial or complete obliteration of the femoral superficial artery with varying degrees of collateral circulation and involvement of proximal or distal arteries. In a number of patients the main lesion was situated in the aorto-iliac vessels or in the popliteal-tibial vessels.

In all the patients the physical examination was completed with ECG, blood counts, ESR,

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blood cholesterol and lipids. Many of the patients had blood cholesterol at the upper border of the normal range (350 mg %) but true hypercholesterolemic patients were excluded. Finally aorto-arteriographic studies were undertaken in all the patients.

Methods

Experimental room. By means of automatic regulation the room temperature could be kept constant within 0.5 C and the humidity at around 50 (4 11 13)

Plethysmography. The blood flow through the calf was estimated by means of venous occlusion plethysmography (1). The calf blood flow at rest is, as will be demonstrated later of little value in assessing the degree of arterial insufficiency. Function tests are therefore employed. In the present work a standard amount of ischemic exercise was used to produce a hyperemia in the calf muscles. The peak flow of this hyperemia is a reliable index for the vascular capacity of the limb. This peak flow is also referred to as the capacity flow.

Postural tests. These tests were as the plethysmography carried out at an ambient temperature of 30°C in order to abolish the sympathetic vasoconstrictor tone (9). After resting in the supine posture for one hour the patient being naked except for a blanket over the genital area, adaptation to the environment was considered complete.

Still with the patient recumbent on a couch the leg was elevated and forceful movements of the foot undertaken by the patient once a second for one minute (18). The extent of plantar ischemia was then noted in arbitrary units as one or two plus or zero. The appearance time of ischemia was not recorded, as initial experiments revealed difficulties in assessing this insidious phenomenon unequivocally.

After having noted the plantar ischemia the limb was hung down over the edge of the couch with the patient still being supine. The time of venous filling on the dorsum of the foot was then recorded in seconds (6).

Simultaneously the reactive hyperemia of the skin on the forefoot was noted and the time of its maximum recorded in seconds (14 15).

Table I. The plantar ischemia test versus the other postural tests and the calf blood flow. Average values from 124 examinations of 60 extremities with intermittent claudication. Absent plantar ischemia in 15% of the examinations. Normal values in brackets

Plant. isch. test	Ven. filling time (secs.)	React. hyp. time (secs.)	Peak flow (ml/min/100 ml)
++	27	37	13.3
+	20	25	14.0
0	13	16	16.7
(0)	(<10)	(<10)	(29.1)

Table II. The postural tests by obstruction of the aorto-iliac, superficial femoral and popliteal-tibial (crural) arteries

Site of main lesion	Plant. isch. test	Ven. filling time (secs.)	React. hyp. time (secs.)	Peak flow (ml/min/100 ml)
Aorto-iliac.	+	18	22	13.7
Femoral art.	+−0	16	21	16.6
Crural art	++	30	36	11.3

Results

The first problem considered was to what extent the plantar ischemia test reflected the capacity blood flow through the calf (table I). Its ability in this respect was poor, plantar ischemia being absent even at a calf flow of 60 % of the normal.

There is an obvious relationship between the three tests indicating that they reflect the same phenomenon. Being distinctly positive also when the plantar ischemia is absent, the tests of venous filling and reactive hyperemia seem the more reliable.

Table III. The spontaneous variation in venous filling time in ten extremities with intermittent claudication in the course of four months

Months				G_e	S. d.	V. c.
1	2	3	4			
15	20	15	15	16	2.5	15.6
35	60	60	30	46	16.0	35.0
15	15	15	15	15	0.0	0.0
25	35	30	35	31	4.8	15.5
15	25	10	15	16	6.3	40.0
15	25	15	15	20	5.7	28.5
15	10	10	10	11	2.5	22.7
20	10	10	15	14	4.8	34.3
15	15	10	10	12.5	2.9	23.2
10	15	30	15	17.5	8.8	50.6
Mean						
19	23	20.5	17.5	19.9	5.4	26.5 %
Monthly var						
19	23	20.5	17.5	20	2.35	11.8 %

G_e = individual mean; S. d. = standard deviation (in the calculation n—1 is used for no. of months) V. c. = coefficient of variation.

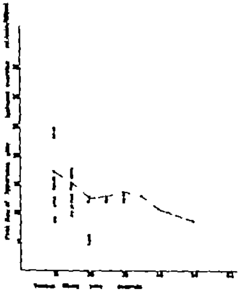


Fig. 1. The relationship between the capacity blood flow of the calf and the venous filling time in intermittent claudication. Venous filling time adjusted to the nearest 5 second interval. Interrupted line drawn through the mean of the flow values for the given times of venous filling.

An important question is whether or not the postural tests could provide information about the site of main artery obstruction (table II). The lowest values are obtained in femoral artery stenosis. An increase is noted in aorto-iliac stenosis, whereas all the tests are strongly positive in crural obstruction.

The relationship between the outcome of the postural tests and the actual limb blood flow seems to be slight in the above tables. This is confirmed in fig. 1 and 2. The venous filling time test is obviously related to the limb blood flow. However, it is hardly possible from a given time for venous filling to predict the blood flow exactly.

Being the best of the postural tests it is important to know whether the venous filling time test is applicable for follow

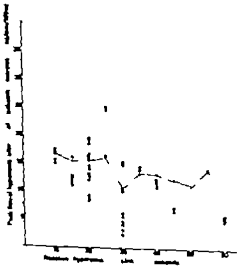


Fig. 2. The relationship between the capacity blood flow of the calf and the reactive hyperemia time in intermittent claudication. Diagram constructed as in fig. 1.

up investigations (table III) However the variation from month to month for the single individual as well as for a group of patients is of such an order that the test becomes of doubtful value in this respect.

Comments

Skin and muscle circulation may behave differently and be differently involved in vascular disorders (10) The postural tests are all confined to the foot which merely reflects the skin circulation. The latter constitutes a minor part of the total limb blood flow the major part being the muscle flow In obliterative arteriosclerosis of the limbs the muscle blood flow is always first and most affected Theoretically the postural tests must therefore be of doubtful significance in assessing the limb blood flow in intermittent claudication. The present day use of the postural tests in the clinical examination of ischemic limbs consequently needs revision.

In this work the results of the postural tests were matched against the muscle blood flow of the calf the latter expressed by the peak flow after a standard amount of ischemic exercise The examined limbs suffered from intermittent claudication and did not present noticeable trophic lesions of the skin.

The widely used test of plantar ischemia on elevation was found to be of little value. Actually it was misleading in femoral artery stenosis as it in many cases was negative in spite of a severe reduction of the total limb flow

The other two tests were both more reliable, the better of them being the venous filling time test, which showed a certain correspondence to the calf blood flow However the range of flow values for a given time of venous filling was so wide that this test could be translated into terms of blood flow only with caution.

In general the time for venous filling was a little shorter than the time for reactive hyperemia in contrast to what has been found by Ratschow (16) This may be due to the by passing of blood from artery to vein in obliterative disease as it is seen by arteriography and as has been reported by Hyman and Winsor (12)

The behaviour of the postural tests in different sites of obstruction was remarkable Their lowest sensitivity was noted in femoral stenosis with a little increase in aorto-iliac stenosis, whereas their greatest degree of positivity occurred in stenosis of the popliteal tibial arteries. These findings are of importance in planning reconstructive surgery for occlusion of the aorto-iliac or femoral arteries. It is well known that involvement of the crural arteries causes a poor drainage which may seriously reduce the benefit of such surgery The same vessels are also as a rule poorly visualized by arteriography However a strongly positive outcome of the postural tests may signal involvement of the crural arteries and be of great help in preoperative considerations

Ratschow (16) has combined the postural tests in his Lagerungsprobe" He finds that the plantar ischemia test usually behaves as in the present work, often being negative in femoral artery obstruction. However according to him the two other tests become increasingly positive when the site of occlusion becomes more and more distal Such a discrepancy between the plantar ischemia test and the two other tests is difficult to explain because all three tests reflect the same phenomenon, namely the skin

circulation of the foot. In the present work there was agreement between all three tests at all levels of obstruction.

Ratschow (16) has expressed his confidence in the postural tests by the following words Ihre Konstanz bei Innenhalten gleicher Bedingungen ist so gross, dass die in Monaten und Jahren beim Kranken erhobenen Befunde einen guten Spiegel der Entwicklung des Leidens abgeben. This statement is not supported by the findings in this report. The variability of the venous filling time from month to month was considerable for the single patient as well as for a group of patients. The venous filling time test seems therefore of limited value for follow-up procedures.

The spontaneous variation of the venous filling time in the course of some months was examined in a group of patients. The variation found was so great that this test is considered unsuitable for follow-up examinations.

Summary

The postural tests for arterial insufficiency have been examined in patients with intermittent claudication.

The result has been compared with the capacity blood flow of the calf as obtained by plethysmography.

All three tests corresponded only poorly with the actual blood flow. The venous filling time test was the best in this respect. It was, however, hardly possible to predict the blood flow from a given time of venous filling.

The plantar ischemia test proved to be the less reliable. It was often negative in femoral artery occlusion with severely reduced capacity blood flow.

The tests were all more positive in aorto-iliac than in femoral artery obstruction. The strongest degree of positivity occurred in obstruction of the crural arteries.

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The Peripheral Blood Flow in Intermittent Claudication

II. The Significance of Skin Thermometry

By

Leif K. Hillestad

Since its application in 1928 (Allen and Smithwick) skin thermometry has been widely used in studies of the peripheral blood circulation. However in latter years several reports have substantially changed the concept of skin thermometry as an index of peripheral blood flow.

Even done under strict control of the environment it does not always provide correct values (11, 12, 29, 31). The thermal lag of recordings, the influence of limb size and evaporation make its interpretation difficult. At large blood flow skin temperature is relatively insensitive, at low blood flow very sensitive.

In fingers Fetcher et al. (16) demonstrated a lack of correspondence between blood flow and skin temperature at 31 C and at 21 C of ambient temperature. Only when the latter was 7°C or lower was the relationship satisfactory. For clinical studies such ambient temperatures are out of question.

In toes Hurni and Simeone (23) confirmed the absence of any relationship between flow and temperature at 20°C. By comparing skin temperatures with arteriography in vascular disease of lower extremities Beckman (8) came to the conclusion that skin thermometry had a restricted value for determination of the site and extent of the obliterations.

The concept of skin and muscle circulation as separate entities (3, 4, 15, 20) and the recognition of the counter current principle (6, 9) have moreover increased the difficulties in translating skin temperatures into terms of blood flow. The precooling of arterial blood may be of special significance for the collateral vessels, which often run more superficially than the main arteries (18).

Finally the observations of Clara (13) and Curri et al. (14) are of great importance. The warm feet in arteriosclerosis may be due to fibrous degeneration of the muscular coat of the arterio-venous anastomoses, leaving them permanently open and with little or no sympathetic control. In keeping with this Hillestad (21, 22) demonstrated resting blood flows above normal in hands with advanced

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obliterative arterial disease. He also showed that under influence of sympathicolytic drugs the blood flow in hands and feet with arterial disease remained unaltered or even decreased in spite of large increases in skin temperatures.

In the present paper the significance of skin thermometry has been evaluated in intermittent claudication of the calf due to arteriosclerosis obliterans. The temperature readings have been matched against the blood flow through the calf muscles as obtained by plethysmography which is considered the best method for recording the peripheral blood flow (8, 19).

For further information about the definition of intermittent claudication and criteria of the patients the reader is referred to a previous communication (22).

Methods

Skin thermometry. This was done by means of thermocouples, made of constantan-copper wires (11, 26), which were joined in a small soldering point and mounted on a little plastic disk. The latter was loosely applied to the skin by means of adhesive tape. The thermocouples operated in a potentiometer circuit with twelve channels and automatic recording device calibrated directly in degrees centigrade (Honeywell Brown Electronic, England).

Longitudinal thermometry of the limbs was not undertaken due to the limited value of the information obtained thereby (6). The temperature of the acres is most sensitive to flow alterations of the limb, in particular after block of sympathetic impulses (1, 5, 19). The temperature was therefore recorded on the anterior plantar surface of the 1st toe. This is also referred to as the skin temperature of the foot.

Muscle thermometry. This was done in a few experiments only. A thermo-needle as designed by Krog (24) was used and care taken that the needle was not close to major arteries during recording.

Tibial posterior nerve block. About 5—10 ml of 1 per cent Xylocaine (Astra, Sweden) was injected around the nerve behind the internal malleolus (37) and the skin temperature recorded for the following 30 minutes. This end point was selected, because the skin temperatures in many cases decreased just beyond this time, as also noted by Beckman (8). The adequacy of the block was easily tested by the subsequent anesthesia of the sole of the foot.

Paravertebral block of the sympathetic nerve impulses is the best procedure, because it includes the whole limb with the collateral vessels (7). The muscle blood flow is little altered by such blocks due to the sparse supply of sympathetic nerves in skeletal muscles (15). However paravertebral blocks are difficult to perform and not suitable for repetition at short intervals. Furthermore the effect of sympathetic block on skin temperature is, as already mentioned, greatest at the periphery of the limb due to the arterio-venous anastomoses and the rich supply of sympathetic nerves. Although paravertebral block gives the greater rise of skin temperature, the latter will be altered in the same direction as by peripheral block. It is therefore probable that the distribution pattern of skin temperatures in a series of extremities would be very much the same, regardless of the method used. This point of view is supported by the extensive use of peripheral nerve block for predicting the outcome of and controlling the result of sympathectomy (23, 39).

Experimental room. This was equipped with automatic control of temperature and humidity (10, 25, 26). During skin thermometry the room temperature was kept at 20°C and the humidity at around 50.

Plethysmography. The calf blood flow was estimated by means of venous occlusion plethysmography (5, 19) which was done at 30 C of room temperature in order to abolish sympathetic vasoconstrictor tone in the skin. For comparative purposes plethysmography was done at 20 C as well as at 30°C in some instances.

The resting calf flow was measured. As this flow value says very little about the capacity flow of the extremity, the calf flow was also studied after a standard amount of ischemic exercise of the calf muscles. The

Table I. The effect of different ambient temperatures on the skin temperature of the foot (1st toe) before and after blockage of the posterior tibial nerve. Average values from two normal extremities. Temperature in degrees centigrade

Room temp.	Initial skin temp.	Skin temp. after nerve block.
25	30	35
20	24	34
15	19	28

Table II. The effect of different ambient temperatures on the skin temperature of the foot before and after blockage of the posterior tibial nerve. Average values from ten extremities with intermittent claudication of the calf. Temperature in degrees centigrade

Room temp.	Initial skin temp.	Skin temp. after nerve block.
25	28	33
22	24	32.5
20	22.5	31.5
18	19	30
15	18	26

Table III. The relationship between skin temperature of the foot and the calf flow. Average values from fifty extremities with intermittent claudication of the calf. Room temperature 20 C. Skin temperature in degrees centigrade. Blood flow in ml/min/100 ml tissue

Skin temp. of the foot	Resting calf blood flow	Peak flow of the calf
21	2.8	17.0
22	3.3	12.0
23	3.5	12.5
24	2.9	16.0
25	2.9	13.0
26	3.3	12.5
27	2.8	10.2

Table IV. The relationship between the skin temperature of the foot after blockage of the posterior tibial nerve and the peak flow. Average values from fifty extremities with intermittent claudication of the calf. Room temperature 20 C. Skin temperature in degrees centigrade. Blood flow in ml/min/100 ml tissue

Skin temp. after nerve block.	Peak flow of the calf
25	14.0
26	19.0
27	7.5
28	14.0
29	10.5
30	13.0
31	17.0
32	17.5
33	15.0

peak flow of the subsequent hyperemia of the calf flow is an index of the capacity flow of the extremity and is used in this paper under the names of peak flow or capacity flow. The detailed description of the plethysmography will appear in a later communication.

Results

The first experiments were done to find the most suitable room temperature for skin temperature studies, (tables I and II)

It is noteworthy that a change in ambient temperature of 5 degrees to each side of 20 C caused about identical changes in normal subjects and in patients. Between 15 C and 25 C of room temperatures the changes in ambient and skin temperatures are disproportionate. This result stresses the importance of keeping a constant room temperature from time to time when skin temperature recordings are to be compared.

The skin temperature after peripheral nerve block was studied to see if it could indicate the limb blood flow and be of help in distinguishing between limbs

obliterative arterial disease. He also showed that under influence of sympathicolytic drugs the blood flow in hands and feet with arterial disease remained unaltered or even decreased in spite of large increases in skin temperatures

In the present paper the significance of skin thermometry has been evaluated in intermittent claudication of the calf due to arteriosclerosis obliterans The temperature readings have been matched against the blood flow through the calf muscles as obtained by plethysmography which is considered the best method for recording the peripheral blood flow (8 19)

For further information about the definition of intermittent claudication and criteria of the patients the reader is referred to a previous communication (22)

Methods

Skin thermometry This was done by means of thermocouples, made of constantan-copper wires (11 26) which were joined in a small soldering point and mounted on a little plastic disk. The latter was loosely applied to the skin by means of adhesive tape The thermocouples operated in a potentiometer circuit with twelve channels and automatic recording device calibrated directly in degrees centigrade (Honeywell Brown Electronic England)

Longitudinal thermometry of the limbs was not undertaken due to the limited value of the information obtained thereby (8) The temperature of the acres is most sensitive to flow alterations of the limb, in particular after block of sympathetic impulses (1 5 19) The temperature was therefore recorded on the anterior plantar surface of the 1st toe This is also referred to as the skin temperature of the foot.

Muscle thermometry This was done in a few experiments only A thermo-needle as designed by Krog (24) was used and care taken that the needle was not close to major arteries during recording

Tibial posterior nerve block. About 5—10 ml of 1 per cent Xylocaine (Astra, Sweden) was injected around the nerve behind the internal malleolus (32) and the skin temperature recorded for the following 30 minutes. This end point was selected, because the skin temperatures in many cases decreased just beyond this time, as also noted by Beckman (8). The adequacy of the block was easily tested by the subsequent anesthesia of the sole of the foot.

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Experimental room. This was equipped with automatic control of temperature and humidity (10 25 26) During skin thermometry the room temperature was kept at 20 C and the humidity at around 50

Plethysmograph The calf blood flow was estimated by means of venous occlusion plethysmography (5 19) which was done at 30 C of room temperature in order to abolish sympathetic vasoconstrictor tone in the skin. For comparative purposes plethysmography was done at 20°C as well as at 30°C in some instances.

The resting calf flow was measured. As this flow value says very little about the capacity flow of the extremity the calf flow was also studied after a standard amount of ischemic exercise of the calf muscles. The

Table VI. The effect of different sites of obstruction on the skin temperature of the foot. Average values from fifty extremities with intermittent claudication. Skin temperature in degrees centigrade. Blood flow in ml/min/100 ml tissue

Site of main lesion	Initial skin temp.	Skin temp. after nerve block.	Peak flow of the calf
Aorto-iliac	22.7	29.5	14.8
Superficial femoral art.	24.3	31.5	15.4
Popliteal-tibial art.	23.0	27.5	10.2

Table VII. Skin temperature of the foot and calf blood flow at room temperatures of 20 C and 30 C in two normal limbs and three limbs with intermittent claudication. Mean increase of skin temperature is 25 and of resting calf flow about 20 %. Mean decrease of peak flow is about 3 %. Skin temperature in degrees centigrade. Blood flow in ml/min/100 ml tissue

Room temp. 20 C			Room temp. 30 C		
Skin temp.	Resting flow	Peak flow	Skin temp.	Resting flow	Peak flow
26.0	2.2	7.8	28.0	2.0	6.2
23.5	1.9	9.4	29.8	2.9	8.5
26.8	1.1	5.4	31.0	1.6	5.1
21.8	2.6	23.3	32.0	3.1	28.8
20.8	3.0	23.3	30.0	3.8	20.5
Mean 23.7	2.1	14.2	30.6	2.7	13.8

(table V) The mean values show a higher ski temperatur in the normal or less affected limb, but this impression is invalidated by a study of the individual records.

If skin temperatures are arranged cor responding to the site of the main b- structive lesions (table VI) they are of

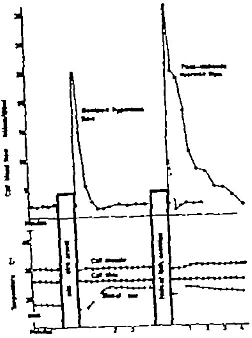


Fig. 2. A comparison between the plethysmographic and the thermometric method in the study of peripheral blood flow. Average data from two normal extremities. Room temperature 25°C.

a certain significance. After nerve block they are normal in femoral artery ste nosis, a little below normal in aorto-iliac stenosis and rather markedly decreased in popliteal-tibial stenosis. There is also a distinct relationship between the capac itive calf flow and the skin temperature after block when arranged according to the site of the obliterative lesions.

In this report skin temperature measurements done at 20°C of room temperature have been compared with plethysmographic blood flows obtained at 30°C of room temperature, and the question is whether this is correct. In some ex periments (table VII) the calf blood flow at the two different ambient temperatures was examined. As expected th resting

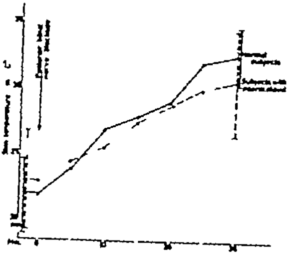


Fig 1 Studies of the skin temperature of the foot (I toe) in twenty extremities with normal blood circulation and twenty extremities with intermittent claudication. The vertical lines at the initial skin temperature and at its end-point after 30 min represent 2 standard deviations to each side of the mean.

Table V The relationship between skin temperature of the foot and the calf flow in both limbs of ten patients with intermittent claudication. Skin temperature in degrees centigrade Blood flow in ml/min/100 ml tissue

Affected limb			Less affected or normal limb		
Skin temp. of foot	Resting flow	Peak flow	Skin temp. of foot	Resting flow	Peak flow
25.0	3.8	14.5	27 0	2.6	23.0
23.0	1 4	5 0	23.0	2.4	20.0
23.0	4.1	9 1	25.0	4.5	18.0
26.2	4.0	15.3	24.2	1.5	22.4
21.3	3.0	4.3	24.0	6.0	7.3
25.0	3.2	21.8	27.5	2.4	34.5
25.5	2.5	3 7	27.0	2.5	7.4
21.5	4.1	9.1	22.5	3.0	11.0
26.0	3.2	14 0	26.5	2.8	18.3
22.0	2.5	20.0	23.0	2.8	31.5
Mean 23.9	3.2	11 7	5.0	3.1	20.1

with normal and pathological circulation. Probably the latter demand would be best met with at the room temperature by which the net increase of skin temperature after block was greatest. It was found to be 18 C to 20 C (table II) Below this border the vascular tone as expressed in the skin temperature seemed to be determined also by other factors than the symphathetic impulses. Above this border the sympathetic tone decreased

An important observation revealed in tables I and II is the identical response to varying ambient temperatures in the normal subjects and the patients This indicates that the vasomotor control is normal in limbs with intermittent claudication, but without notable trophic disturbances.

The ability of skin temperature readings in providing informations on the limb blood flow showed up to be a matter of disappointment (tables III and IV) Even by a 70 % reduction of the capacity flow the skin temperature could be as high as 27 C Similarly the skin temperature after block reached 33 C (normal level see below) in spite of a 50 % reduction of the capacity flow

These results are supported by the experiments illustrated in fig 1 The normal range of skin temperatures before as well as after nerve block is so great that there must be a considerable overlapping between normal and pathological cases

The mean normal skin temperature after block is 32 5 C. If two standard deviations of 1 05 each are subtracted the lower normal border becomes 30 4 C. This is identical with the findings of 30 5 C by Morton and Scott (28)

According to the above results it is no surprise that skin thermometry could not indicate the affected or the most affected limb of two belonging to the same patient

is probable that the lower obstruction more directly affects the inflow to the foot.

A similar response was also obtained with the postural tests (22). The latter and the skin temperature after block have in common that they are confined to a foot, which is in a state of vasodilatation. It may therefore be concluded that a strongly positive outcome of the postural tests or a poor response of skin temperature of the foot after nerve block indicates obliterative changes in the popliteal-tibial arteries. The simple postural tests may thus replace the skin thermometry.

The old assumption that arterial insufficiency of a limb is accompanied by reduced temperature of skin (30) is not correct (18) according to the present report. Neither is it possible by means of skin temperature readings to point out the most affected of two limbs in the same individual. The studies of skin temperatures in normal and pathological limbs before and after peripheral nerve block and the illustrated comparison between thermometry and plethysmography likewise support the above results.

The conclusion is that skin thermometry has a restricted value in the examination of limbs with intermittent claudication and without notable trophic lesions of the skin.

Summary

The significance of skin thermometry in intermittent claudication has been evaluated. The skin temperatures of the feet before and after peripheral sympathetic block were matched against the calf blood flow as obtained by means of plethysmography. The calf blood flow at rest as well as after a standard amount of ischemic exercise was measured.

Preliminary experiments supported the demand for strict control of ambient temperature in studies of skin temperature. The most suitable room temperature for recording the effect of sympathetic block was found to be 18 to 20°C. The reactions of skin temperature to varying environmental temperatures indicated a normal sympathetic vasomotor control in the limbs with intermittent claudication.

No obvious relationship could be demonstrated between the skin temperature and the calf blood flow at rest or after ischemic exercise. Actually the skin temperatures after nerve block could be misleading with regard to blood flow and collateral circulation.

The skin temperatures after block were often normal in femoral artery stenosis, a little decreased in aorto-iliac stenosis, but severely decreased in popliteal-tibial stenosis. The significance of these results are discussed.

When the room temperature was changed from 20°C to 30 C, the resting flow of the calf and the skin temperature of the foot increased by the same amount, namely about 25 %. At the same time the calf flow after 1 minute of ischemic exercise decreased by about 5

Experiments showed that there is a considerable overlapping of skin temperatures before as well as after nerve block in limbs with normal and pathological arterial circulation. Likewise the superiority of plethysmography contra thermometry in assessing the peripheral blood flow is illustrated by some experiments.

According to the present study skin thermometry has a limited value in the examination of limbs with intermittent claudication.

flow increases a little at the higher room temperature due to release of the vasoconstrictor tone in the skin of the calf whereas the muscle flow decreased a little (3 4 15 20) The changes are, however hardly so great that the comparisons between calf flow and skin temperature of this report are incorrect. Moreover the flow values are used to characterize the limb and a limb is thus normal or pathological regardless of the ambient temperature.

Finally a comparison between the methods of thermometry and plethysmography is illustrated in fig 2 The lag period of thermometry is obvious. Typical also is the lacking response in skin temperature of the calf whereas the skin temperature of the foot shows a considerable rise. At the right upper quadrant of the figure the hyperemic flow after 1 minute of ischemic exercise is depicted At the bottom of this curve the hyperemic flow after 1 minute of ischemia without exercise is drawn. The area above the latter curve indicates the large flow which is exclusively attributed to the calf muscles In contrast to this large muscle blood flow stands the negligible change of muscle temperature.

Comments

The introductory remarks of this paper reviewed the many difficulties encountered in skin thermometry For most of them no allowances can be made, a fact which must restrict the value of skin thermometry This view is fully supported by the present report.

It has been demonstrated that the skin temperature of the foot neither before nor after peripheral sympathetic nerve block bears any relation to the calf blood flow at rest or after muscular exercise. This means that at room temperatures ordinarily used there is no relationship between the skin temperatures and the circulation through the skin (16, 23) or the muscles in the lower extremities.

Moreover skin thermometry may provide misleading data. As also demonstrated by others (17 18) the skin temperature after nerve block may reach normal values in the presence of severe arterial insufficiency This has been attributed to a collateral circulation, which may compensate the arterial stenosis (8, 17) The plethysmographic studies of blood flow in the present report contradict this explanation as the only one. The plethysmographic studies of blood flow includes also the collateral flow Several limbs in the present series with capacitive calf flow between 17.5 and 15 0 ml/min/100 ml showed a normal temperature response after nerve block. If fully compensated the recorded flows would have been at the normal level around 29 ml/min/100 ml The skin temperature after nerve block is therefore not determined only by the capacitive blood flow and the extent of collateral circulation.

As demonstrated the level of stenosis is more significant in this respect. The lowest temperature response after block occurred in stenosis of the popliteal tibial arteries, whereas the response was normal in femoral artery stenosis and only a little below normal in aorto-iliac stenosis That obliteration of the crural arteries had a great influence was also found by Beekman (8) He explained this by the existence of bridging collaterals in high obstruction and by absence of the same in low obstructions (27) This view is not supported by this report, because the differences in blood flow between the three levels of obstruction were small. It

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From Troms & Tromsö Hospital, Tromsø Norway

Macroglobulinemia Waldenström

A Case with Unusual Findings

By

Sverre Nordöy

Essential macroglobulinemia was originally described by Waldenström in 1944 (38). Later several cases have been reported, and detailed surveys have been given by Schulten & Kanzow (32), Kappeler et al. (11), Waldenström (39) and Ritzmann et al. (24). From Norway one case has been reported by Alvsaker (1).

The course of the disease and the results of electrophoretic, ultracentrifugal and immunological investigations vary in different cases. In one case of macroglobulinemia Waldenström most of the bone marrow cells contained nuclear inclusions, and ultracentrifugation of the patient's serum revealed unusual heavy protein fractions.

Case report

A 67-year-old man was admitted to the medical department, Troms & Tromsø Hospital, in July 1958 on account of anemia and high sedimentation rate. Besides the pallor the only abnormal findings on clinical examination were moderately enlarged lymph nodes. Hemoglobin 62 %, erythrocytes 3.21 mill./mm³, colour index 0.97, leukocytes 10,000/mm³, ESR 98 mm/h. Bleeding time 4 min., coagulation time 4 1/2 min. Examination of the urine revealed albuminuria, but no trace of Bence-Jones protein was found. X-ray examination of the skeleton showed normal bone structure. In the peripheral blood, abundant abnormal cells were found, their size varied between 7 and 20 microns. The cells were suggestive of paramyeloblasts or monocytoid myelocytes. In the bone marrow cells resembling plasma cells and small, lymphocyte-like cells dominated. The picture was suggestive of paramyeloblast leukemia (35).

Before further examinations could be carried out, the patient was discharged on his own request.

In April 1960 he was readmitted to the medical department because of increasing anemia. He was extremely pale and on clinical examination general edema, and moderately enlarged lymph nodes, spleen and liver was detected. X-ray examinations and electrocardiograms gave no evidence in support of cardiac or pulmonary disease. Hemoglobin 37 %, erythrocytes 1.9 mill/mm³, colour index 1, leukocytes 11,300/mm³; differential: many large, lymphocyte-like cells were seen. There was marked rouleaux formation of the erythrocytes. Thrombocytes 123,000/mm³, prothrombin-proconvertin time (PP a. m. Owren) 80 %, bleeding time 3 min. The fragility of the erythrocytes was normal, Coombs' direct and indirect tests

Submitted for publication February 14, 1962.

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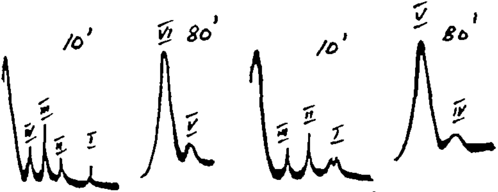
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b

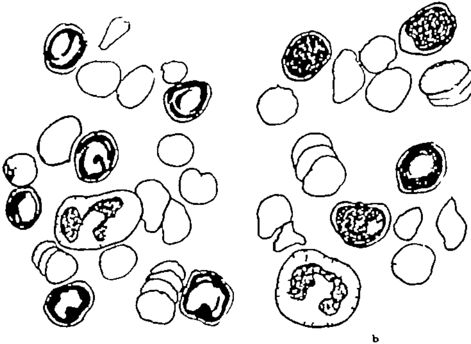
Fig. 2. Ultracentrifuge diagrams (time in min.).

a) 27/6-60.

I.	S = 33	rel. conc.	2.0 %
II.	S = 26		4.5 %
III.	S = 17		10.9 %
IV	S = 12		6.9 %
V	S = 7.2		2.5 %
VI.	S = 4.3		74.3 %

b) 17/9-60

I.	S = 18.5	rel. conc.	5.3 %
II.	S = 14.6		7.8 %
III.	S = 11.5		7.4 %
IV	S = 7.3		6.9 %
V	S = 4.2		72.6



b

Fig. 3. Bone-marrow aspirate (drawing from the microscope picture)

) June 1960. Dominance of the small lymphocyte-like cells, containing nuclear inclusions.

b) Sept. 1960. The nuclear inclusions are reduced in size and number

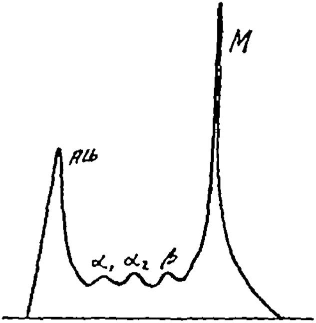


Fig 1 Filter-paper-electrophoresis of serum 18/6-60.
Total protein 10.5 g/100 ml.
Albumin 25 6 (2.69 g/100 ml)
Globulins α_1 5 1 α 8.3 β 7 4 „
γ 53 6 (5.63 g/100 ml)

negative, ESR 156 mm/h. Paper-electrophoresis revealed a narrow high peak in the γ-position. Total serum protein 10.5 g/100 ml (fig 1) Thymol turbidity test, Takata Ara and formol-gel reactions were strongly positive Examination of the urine revealed positive tests for albumin and for Bence Jones protein.

The bone marrow was dominated by uniform looking cells with scarce basophil cytoplasm without characterist c features. The nuclei were relatively large and the cells looked l ke small lymphocytes. Most of the cells conta ned spherical, homogenous, nuclear inclusions, the chromatin of the nuclei being displaced to the periphery like dark, circular or semi-circular bands (fig 3 a) The diagnosis of macroglobulinemia Waldenström was made.

During the hospitalization the patient suffered from nose bleeding several times. Several blood-transfusions were given and his condition grad ally improved He went home and was readmitted to the department one month later in poor condition, hemoglobin 45 thrombocytes 78,000/mm PP 50 total serum cholesterol 83 mg/100 ml (Bloor) Ultracentrifugation of the patient's serum was performed at the Carlsberg Bryggeriers Forsöksläboratorium in Copenhagen, Denmark. Of the serum proteins, 16.3 % were macroglobulins, and two of the fractions had unusually high sedimentation constants (Svedberg units) 26 and 35 S, respectively (fig 2 a) More blood transfusions were given, the patient was discharged in better condition but was admitted to the medical department one month later with anemia general edema and fever ESR as 147 mm/h and paper-electrophoresis and bone marrow examinations revealed unchanged conditions. In the ultracentrifugal pattern the heaviest component had disappeared. After more blood-transfusions, treatment with prednisone was started. At a dose of 15 mg daily the patient's condition rapidly improved, particularly as regards the hemorrhagic diathesis.

In Sept. 1960 his condition was relatively good Ultracentrifugal examinations revealed a decreased content of macroglobulins, and the sedimentation constants of the fractions had changed (fig 2 b) The number of the nuclear inclusions in the bone marrow cells had decreased considerably There was no hemorrhagic diathesis, but the enlargement of the lymph nodes, spleen and liver remained unchanged The patient has taken prednisone ever since and apart from the anemia, which requires blood-transfusions at several months interval, he manages fairly well.

Discussion

The *macroglobulins* are gluco-lipoproteins (31) Normally they account for approximately 2 % (rarely exceeds

nuclear material was dislodged to the periphery of the nuclei as dark bands. At last, the inclusions passed through the nuclear membrane and the cytoplasm to the intercellular space where they formed free globules. The nuclear inclusions, the intercellular globules, the "protein-lakes" and the granules in the large histiocytes of the bone marrow histochemically consist of glucoproteins, giving a positive PAS-reaction (4 15 16). Presumably this glucoprotein is identical with the macroglobulin in macroglobulinemia Waldenström (4,27) and it is believed that the macroglobulin is produced by the lymphocyte-like cells (11 15 19).

The number of cells containing nuclear inclusions are sparse in the reported cases. In the present case, the bone marrow was flooded by such cells before treatment (fig. 3 a). After 6 weeks of treatment with prednisone, the bone marrow was more normal looking, the nuclear inclusions had decreased considerably in size and number and the heaviest macroglobulin components had disappeared from the serum. It is not known if these changes were due to the treatment or a spontaneous emission alone. As the patient' general condition improved markedly at the same time, t is just possible that there was a relationship between the clinical picture, the cellular changes in the bone marrow and the molecular weight of the macroglobulins in this case.

The diagnosis of macroglobulinemia Waldenström is made by the clinical symptoms, the accelerated sedimentation rate and abnormal serological tests, the paper-electrophoretic pattern and bone marrow examination. In doubtful cases, ultracentrifugal diagrams and the result of immunological tests will be decisive. In some cases, a clear-cut diagnosis of macroglobulinemia Waldenström can be extremely difficult to make, and it is presumed that intermediate forms exist (40). In certain cases, an increased number of plasma-cells in the bone marrow have been described (13 37 43). Some authors have observed transitional forms between macroglobulinemia and multiple myelomas, and they think that macroglobulinemia Waldenström is a particular type of multiple myeloma (27 28 29). In the present case, an increased number of plasmacell-like cells and lymphocyte like cells were found side by side in the bone marrow two years before the diagnosis was made. There was no trace of Bence Jones protein in the urine then. In 1960 the plasma-cell type of cell had disappeared from the bone marrow but now examination of the urine revealed a positive test for Bence-Jones protein. Because of these findings it is just possible that this case represent an intermediate form. Overlapping also between macroglobulinemia Walden strom and lymphatic leukemia (17 41) and between macroglobulinemia and reticulosarcoma (15 16 43) have been described.

The etiology of the disease is not known, neither is any fundamental treatment. Corticosteroids and ACTH as a rule only produce transient relief (9 11 24 41). In the present case, the prednisone treatment seemed to be of benefit. Repeated bloodtransfusions are necessary as in the present case but often utoimmune-antibodies and severe transfusion-reactions appear during treatment. Schwab et al. attained prolonged remission in a case of macroglobulinemia Waldenström by plasmaphoresis (33). In 1957 Deutsch and Morton found that ddition of sulphhydryl-groups (mercaptans) resulted in the disappearance

5 %) of the total serum proteins (21) An increase in the fraction up to 15 % may be seen in cases of neoplasm, collagen disease and infection (24) In the primary idiopathic type of macroglobulinemia the fraction exceeds 15 % of the total serum proteins. In the present case the M fraction amounted to 16 3 %

The molecular weight of the macroglobulins may be as high as 1 800 000 as compared with 160 000 of the normal gamma globulins and 35 000 of the Bence Jones protein (24) The sedimentation constant S (Svedberg unit) is consequently high The S-value of normal gammaglobulins is approximately 7.5 as compared with a S-value of more than 15 of unequivocal cases of macroglobulinemia Waldenström. The tiny M fraction has the characteristic S-value of approximately 20 but in rare cases values up to 32 have been described (18 23) It is difficult to compare the S-values of the various previously reported cases, because the concentration of the serum proteins may influence the sedimentation properties (12) In addition the sedimentation constant alters when a macroglobulin is isolated, purified and ultracentrifuged anew (9) S-values of 35 to 40 have been described for such isolated macroglobulins (2) In the present case the serum contained small fractions (not purified) of unusual heavy macroglobulins (S 26 S 35) One cannot set aside the possibility however that the long transport from Tromsö to Copenhagen had influenced the aggregation properties of the macroglobulins in some way or the other

In the paperelectrophoretic pattern the macroglobulin forms a narrow high peak in the globulin fraction. Most frequently the M peak is found in the gamma position, but often also in the beta$_2$ — and phi — (fibrinogen-)position. The distinction between macroglobulinemia Waldenström and multiple myeloma cannot be based upon the electrophoretic pattern alone (34) For this purpose, the lately developed immunological tests can be used, because the pathological macroglobulins in cases of macroglobulinemia Waldenström contain individually characteristic and specific antigen determinations, which enables one to distinguish between macroglobulins and myeloma globulins, as well as the primary and secondary types of macroglobulinemia (7 8 30)

In macroglobulinemia Waldenström, the outstanding feature is the presence of lymphoid or lymphoreticular cells in the bone marrow lymph nodes and other organs. The cells are small and resemble mature lymphocytes. As a rule, the cytoplasm of the cells is very scanty basophilic and lacks granulation. Frequently it contains PAS- (periodic-acid-Schiff) staining material sometimes in the form of globules ("grape cells) (24) The nuclei are irregular with a rather coarse chromatin pattern, and the nuclear membrane is sparse (4) Other cells are larger with a relatively scanty deep blue-lilac cytoplasm.

Most authors maintain that the pathological cells are lymphocyte-like reticular cells (6 11 14 26 32) others that they are aberrant lymphocytes (5 20 37)

Intra and inter-cellular deposits of protein in bone marrow lymph nodes, spleen and liver have been described in several cases of macroglobulinemia Waldenström (4 10 15 16 19 27 28,29 36 42) Dutcher & Fahey (4) demonstrated the rise and growth of homogenous or sparsely granulated spherical nuclear inclusions in the lymphocyte-like cells. As the inclusions increased in size, the

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Summary

A case of macroglobulinemia Waldenström is presented because of its unusual feature. Of the total serum protein, 16 3 % were macroglobulins, and two of the fractions had an unusually high sedimentation constant 26 S and 35 S respectively The bone marrow was flooded by small lymphocyte-like cells, and most of the cells contained large nuclear inclusions.

Prednisone therapy seemed to be of benefit to the patient the general condition improved the size and number of the nuclear inclusions decreased and the heaviest protein components disappeared from the serum

A short survey of the literature is given.

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The Association of Acute Benign Pericarditis in Adults with Coxsackie B-5 Virus Infection

By

PER HEDLUND ERIK LYCKE and GÖSTA TIBBLIN

In one of the earliest reports on pleurodynia or Bornholm disease (Bacher 1896 (1)) pericarditis was mentioned as a complication observed. Later other authors were also to report the occurrence of pericarditis during epidemics of pleurodynia. Since methods for virological examination became available for etiological studies, evidence of a connection between acute benign pericarditis and Coxsackie group B virus infections has been brought to light. Thus Bain et al. (2) eported seven cases of children with pericarditis. Coxsackie viruses belonging to type B-5 were isolated from stool samples, and paired serum specimens showed a rise in homologous antibody titers. From adults with pericarditis, Coxsackie virus (type B-5) has been re covered in one case only (3) In an additional two cases the serological findings have indicated Coxsackie B virus infections (4 5) The present report deals with the clinical findings of six cases of acute benign pericarditis and the results of virological examinations indicating Coxsackie B-5 virus as the etiologic agent.

Material and methods

During the summer and fall of 1961 Coxsackie B-5 virus infections were prevalent in many parts of Sweden. The epidemic was characterized by pleurodynia and aseptic meningitis. From the beginning of July till the end of November a total of 139 strains of Coxsackie B-5 virus were isolated at the Virological Laboratory Gothenburg.

In a few patients hospitalized during the period August—October precordial pains dominated the clinical picture. Of these, six patients who had developed typical signs of pericarditis were examined virologically With the exception of a boy aged six, the patients were adults, ages ranging from 24 to 62 years (table I)

Clinical findings

The onset of illness, one to three weeks before admission to the hospital, was characterized by fever Three of the patients also complained of sore throat. The fever followed diphasic course and at the second fever peak precordial pain ppeared. Two of the patients also complained of pain of pleuritic nature.

On admission pericardial rub was heard in five of the patients. This was verified phonographically in three cases. Enlargement of the cardiac shadow was registered in four patients. Normalization was attained a week later The

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trains. Only with two fecal specimens could the virus be recovered in suckling mice primarily. However when the strains isolated in cultures were passaged in suckling mice they caused symptoms typical of Coxsackie B-virus infections.

The results of the neutralization tests are summarized in table II. As will be seen from the table a significant rise in antibody titer was demonstrable in three cases. In the other three cases high antibody levels were already found on admittance but these patients had been ill for more than two weeks.

Table II. Coxsackie B-5 neutralizing antibodies. Titers are expressed as reciprocals of neutralizing serum dilutions

Patient	Serum specimen number		
	1	2	3
E. G.	20	80	160
R. O.	240	320	Not done
M. S.	30	160	480
C. S.	160	240	480
A. C.	240	480	Not done
A. M.	240	480	Not done

Comments

Although available materials are small they demonstrate the association of acute benign pericarditis and Coxsackie B-virus infections. In the present investigation the viral studies indicated Coxsackie B-5 virus as the etiological agent. Isolation of virus from the pericardial fluid which would have supported the demonstration of the etiology was not attempted. Bain et al. (2) failed to isolate virus from a single pericardial fluid obtained. This was derived from a patient who also had pleurodynia but did not excrete virus in the feces and had an antibody titer in the serum of 2,500 as early as eight days after the onset of disease. The isolation of Coxsackie B-3 virus from the pericardial fluid as well as from stool specimens has been reported by Kagan and Bernkopf (6) in a ten-month-old girl with pericarditis.

Two of our patients showed leukocyte counts of more than 10 000/mm³ and elevated antistreptolysin O titers were observed in three cases. β-hemolytic streptococci were found in throat swabs from the six-year-old boy. The possibility that two of the patients also had bacterial complications therefore cannot be disregarded.

The frequency of pericarditis during epidemics of Coxsackie B-viruses is probably higher than indicated by available reports. The disease has mainly been observed among children. Five of the six cases in the present report, however, were adults and one was admitted to hospital under a preliminary diagnosis of heart infarct.

Summary

The clinical findings for six patients with acute benign pericarditis are presented. Five of these were adults. The viral studies showed that the patients were Coxsackie B-5 excretors and developed homologous antibody titers in serum.

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Table 1 Clinical aspects of pericarditis in six patients

Patient	Age sex	Date of admission	Fever	Pericardial rub	Pain	Appearance	ESR (mm/hr) (max.)	Leukocyte count (per mm) (max.)	ASO titer (units) (max.)	ECG findings	Treatment and course
E. G.	39 M	10/10/61	37.8 C	+	Precordial	Not ill	30	6,700	800	Normal	Aspirin
R. O	28 M	18/ 8/61	38.0 C	+	Severe precordial	Ill	54	21,800	1 600	T-wave changes	Aspirin + prednisolone
M. S.	40 F	26/ 8/61	38.0 C	+	Precordial	Not ill	80	—	800	T-wave changes	Aspirin
C. S	6 M	25/ 9 61	38.5 C	+	Precordial	Not ill	59	13,500	200	T wave changes	Penicillin
A. C.	24 M	11 10/61	39.. C	—	Precordial	Not ill	40	8,600	400	T wave changes	No drug treatment Bed rest
A. M.	62 F	10/10 61	38.0 C	+	Precordial	Not ill	50	5,000	—	T wave changes	Prednisolone

ECG of five patients showed T-wave changes indicating pericarditis.

The ESR was elevated in all cases. In one case the child β-hemolytic streptococci were cultured from throat swabs. This patient and one of the others showed leukocyte counts of more than ten thousand. The antistreptolysin titers were elevated in three cases.

No drug treatment was given three patients, whereas two received cortisone because of progression of pericardial effusion. In one of the latter cases signs of cardial tamponade appeared before administration of cortisone. All the patients made a complete recovery within a few weeks of admission to the hospital. Certain of the clinical data are summarized in table I.

Virological examinations

Stool and blood specimens were collected from the patients during the first 48 hours after admission. A second blood sample was drawn two to three weeks later and in certain cases a third blood sample was taken a week after the second.

The stool specimens were tested in HeLa cell cultures and in cultures of trypsinized monkey kidney cells cultivated in a medium consisting of Hanks balanced salt solution, 0.5 lactalbumin hydrolysate 7 calf serum, 100 I U penicillin and 100 γ streptomycin, as well as in one-day old suckling Swiss albino mice. Isolated virus strains were typed in HeLa cell cultures against Coxsackie B antisera prepared by hyperimmunization of mice.

Neutralization tests were performed using 100 $TCID_{50}$ of type B-5 virus and patient serum diluted in twofold steps beginning with 1/20 and 1/30. The mixtures of virus and serum dilutions were allowed to react at room temperature for one hour and then portions of each mixture were tested in two cell cultures. Neutralization was considered to occur if the cytopathogenic effect of virus was neutralized in one of the two test tubes.

Results

Cytopathogenic agents were isolated from the cell cultures of stool specimens of all six patients. In all cases the isolated agents were typed as Coxsackie B-5

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The Renal Excretion of Citric Acid

Preliminary Report

By

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In the past 20 years much research has been done to elucidate the action of the parathyroid hormone on citrate metabolism (5,15). These studies, together with investigations with isotopes, have delineated the significance of citric acid in the function of bone matrix (13). Vitamin D (3 8) a. o. oestrogens (4) a. o., potassium deficiency (14) and a series of endocrine diseases (1 14) also interfere with the metabolism of citric acid. I several of these investigations the conclusions are based only on the citric acid concentration in the blood. Some authors have mentioned the desirability of more accurate investigation of the renal excretion of citric acid. We, therefore, in the Medical Department B Rikshospitalet, have taken up some of the problems of the renal excretion of citric acid.

Previous investigations of the renal excretion of citric acid have concentrated chiefly on the 24 hour excretion in relation to the pH of the urine. Östberg and Sherman et al. (18 16) showed that the excretion of citric acid is reduced in acidosis and increased in alkalosis.

Citric acid makes up a small fraction (4 to 10 % (2)) of the organic acids found in urine. The 24-hour excretion of citric acid normally varies from 200 to 1,000 mg and shows small fluctuations on a constant diet. The same is true of the concentration of citric acid in the blood. It is presumed that citric acid is completely filterable in the glomeruli (10). Besides the glomerular filtration there is tubular synthesis and excretion (11). In experiments with dogs involving catheterization of the renal vein and infusion of buffered citric acid solution it has been shown that the ability of the kidneys to excrete citric acid is very great (11). Normally citric acid does not accumulate in the tubules (10) and Milne et al. (14) assume that there is a dynamic equilibrium between the citrate in the lumen of the tubules and that in the cells of the tubules.

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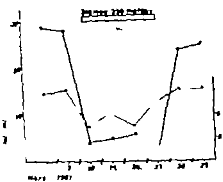


Fig. 3. Endogenous citric acid clearance (C_{cit}) and urinary pH ( ) during treatment with Diamox.

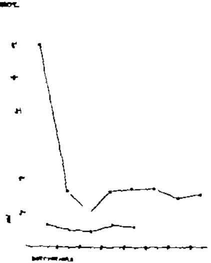


Fig. 4. Endogenous citric acid clearance (C_{cit}) in nephritis glomerulonephritis pyelonephritis.

phritis the C_{cit} fell in the acute phase of the disease and remained low during the chronic phase.

Studies of the quantitative histo-chemistry of the nephron show that the enzyme activity is altered in nephritis. The reduction of C_{cit} values in nephritis recorded in our experiments must probably be attributed to reduced tubular synthesis of citric acid. This is confirmed by our observations in patient with chronic nephritis (type II). The C_{cit} alone remained unchanged during treatment with Diamox (fig. 5).

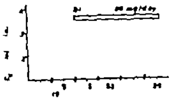


Fig. 5. Endogenous citric acid clearance (C_{cit}) in patient with chronic glomerulonephritis.

Discussion

It has previously been maintained that the 24-hour excretion of citric acid varies with the pH of the urine (18, 16). Endogenous C_{cit} did not vary with the urinary pH in our observations, and Clarke et al. (2) also found no correlation between the excretion of citric acid and the pH in the urine. Our investigations confirm the assumption that it is the intracellular pH that is decisive in the synthesis of citric acid. Diamox causes metabolic acidosis. The equilibrium constant for the enzymatic synthesis of citric acid depends on the pH (17) and is extremely sensitive to variations in pH. The reduction of C_{cit} when Diamox is administered is probably to be explained by reduced tubular synthesis. In all the observations in our tests the serum citric acid was normal and the glomerular filtration (assessed by endogenous creatinine clearance) was unchanged during the test periods. One can therefore exclude variations in the amount of filtered citric acid as a causative factor in the reduction of C_{cit} during administration of Diamox.

It has been debated whether the reduction of endogenous C_{cit} as a result of administration of Diamox might depend on increased reabsorption of filtered citric acid. It is not possible for us to estimate the basal reabsorption of citric acid in the clinic. If the synthesis of citric

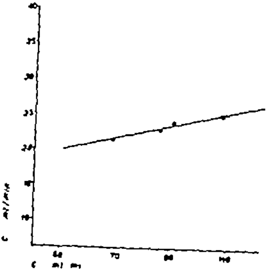


Fig. 1. Scatter graph of endogenous citric acid clearance (C_{cit}) and endogenous creatinine clearance (C_{cr}).

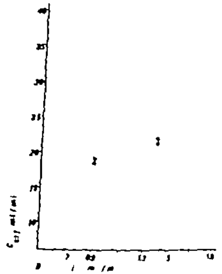


Fig. 2. Scatter graph of endogenous citric acid clearance (C_{cit}) and diuresis.

Present investigations

The citric acid in serum and urine was estimated using the pentabromacetone method. We have used the usual clearance method with 6-hour periods to study the renal excretion of citric acid.

With a constant diet we found small variations in the fasting concentration of citric acid in serum in 30 patients. The average concentration was 2.05 mg/100 ml (± 0.26).

In 10 cases with normal renal function 25 clearance tests were carried out and these showed small variations of the clearance values in the individual cases. The arithmetical mean for endogenous citric acid clearance (C_{cit}) was 3.4 ml/min and the standard deviation 6.4. In dogs the C_{cit} varies from 1.5 to 8.2 ml/min and the ratio C_{cit}/endogenous creatinin clearance varies from 0.03 to 0.118 (12). The clearance ratio in our experiments was 0.267 ± 0.075. In fig. 1 the endogenous C_{cit} is plotted against the glomerular filtration (endogenous creatinine clearance). The correlation coefficient (0.209) is not significantly different from nought. In our investigation there was no correlation between the glomerular filtration and the endogenous C_{cit}.

The influence of diuresis on the endogenous C_{cit} was investigated statistically. There was no correlation between diuresis and endogenous C_{cit} (correlation coefficient 0.09) which shows that endogenous C_{cit} is independent of diuresis (fig. 2).

The synthesis of citric acid is an enzymatic process, and condensing enzyme has been isolated. Citrate is synthetized from acetyl-coenzyme A and oxalacetate (17). In animal experiments it has been shown that several chemical substances block the synthesis and lead to reduced excretion of citric acid in the urine (7). Diamox is the most suitable inhibitor in clinical tests. In 9 cases with normal renal function the endogenous C_{cit} was estimated before, during and after blockade with Diamox. An identical response was found in all the cases, and the observations from one patient are shown in fig. 3. There was a marked fall of endogenous C_{cit} two days after starting the drug and the clearance values rose to the normal level after stopping the drug. There was no parallelism between the clearance values and the pH of the urine.

Endogenous C_{cit} is reduced in different types of nephritis. Fig. 4 shows the observations in one patient with chronic pyelonephritis and in one patient with glomerulonephritis that became chronic. Throughout the observation period the C_{cit} values remained constant in the case with pyelonephritis, but in the patient with glomerulone-

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Etude comparative du thrombotest, du temps de Quick, du test de tolérance à l'héparine et du facteur Stuart

Par

H. Tsevrenis, T. Mandalaki, A. Stavoulidis et J. Joannidis

L'expérience acquise ces dernières années sur le traitement par les antivitamines K a montré que celles-ci constituent encore le seul traitement de base de tout incident thrombo-embolique.

L'efficacité de ce traitement dépend essentiellement des méthodes de laboratoire utilisées pour son contrôle. Toutefois, parmi ces méthodes, les unes sont vraiment excellentes mais délicates et difficilement applicables en grande série et les autres, dont le temps de Quick qui est le plus couramment utilisé, ne reflètent que de loin les modifications réelles de la coagulabilité sanguine provoquées par les antivitamines K. Le thrombotest proposé par Owren semble présenter les deux grandes qualités d'une bonne méthode de contrôle : la facilité d'exécution et la sensibilité aux facteurs de la coagulation influencés par les antivitamines K. Afin de confirmer la valeur de ce test, nous avons étudié, parallèlement à celui-ci, le temps de Quick, le test de tolérance à l'héparine et le taux du facteur Stuart (facteur X). Tous ces tests ont été exécutés simultanément, lors de chacun des contrôles pratiqués. Ceci nous a permis de comparer la valeur réelle de chacun de ces tests devant des incidents hémorragiques rencontrés en cours de traitement et de vérifier les qualités du thrombotest d'Owren.

Méthodes

Temps de Quick. La thromboplastine préparée à partir de cerveau humain était toujours la même pour tous les examens pratiqués.

Test de tolérance à l'héparine. Technique de Soulier et Le Bolloch (9).

Facteur Stuart. (Facteur X) Dosage selon Bachmann, Duckert et Koller (6).

Thrombotest. Sur plasma oxalaté. La technique était celle préconisée par Owren. Le sang était prélevé toujours sur mélange oxalaté de Wintrobe. La solution du réactif (Nyegaard[1]) se conserve parfaitement pendant un temps assez long à —20° C.

Les temps de Quick, du facteur X et du thrombotest, ont été convertis en taux, au moyen d'une courbe établie par dilution du plasma dans une solution de sérum physiologique.

Nombre total de mesures : 225 × 4 = 900.

Nous devons remercier Monsieur Christophore Pallidis et la Maison Nyegaard à Oslo, pour avoir mis à notre disposition les échantillons du réactif thrombotest utilisé pour ce travail.

Ce travail est parvenu à la rédaction le 1 Mars 1962.

acid is completely blocked and *reabsorption not affected* it is possible to calculate the tubular reabsorption of citric acid in the Diamox tests Making these assumptions we have calculated the tubular reabsorption of citric acid in our Diamox tests in 9 cases. The average reabsorption was 90 %, varying from 80 to 97 % with small individual variations in the observation periods

In as yet unfinished investigations entailing catheterization of the renal vein we have studied the renal metabolism of citric acid during administration of Diamox These studies show that Diamox does not increase the tubular reabsorption of citric acid

The citric acid content of the urine is of significance in the prevention of precipitation of Ca in the tubules (6) Calcium has a considerable citrate binding capacity and the relatively undissociated and soluble Ca-citrate complex in the urine does not precipitate The dissociation of Ca-citrate has been clarified by Hastings et al (9) At pH 7 in the urine 60 % of the calcium will be present as Ca-citrate at pH 5 40 % (4) In experiments with rats where the excretion of citrate in the urine is reduced by administration of Diamox precipitation of Ca in the tubules has been demonstrated histologically (7)

Summary

In cases with normal renal function the endogenous citric acid clearance ($C_{cit.}$) was 23 4 ml min and the standard deviation 6 4 There was correlation neither between the glomerular filtration (endogenous creatinine clearance) and $C_{cit.}$ nor between diuresis and $C_{cit.}$

On administration of Diamox the $C_{cit.}$ values were reduced. This is attributed to reduced tubular synthesis of citric acid on account of metabolic acidosis. Assuming that the tubular synthesis is completely blocked and the reabsorption of citric acid not affected by Diamox, the tubular reabsorption of citric acid has been calculated It varied from 80 to 97 % with an average of 90 %.

The $C_{cit.}$ values were reduced in different types of nephritis. This is attributed to reduced enzymatic activity in the tubules.

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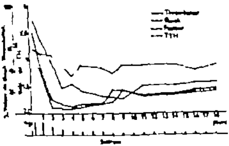


Fig. 2. Aux premiers jours d'un traitement par les antivitamines K, il existe une discordance entre les valeurs données par le thrombotest et les temps de Quick et celles d T.T.H. et du facteur X.

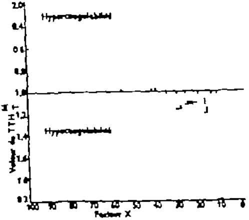


Fig. 3. Parallélisme entre les valeurs du facteur X et du T.T.H.

rence entre le temps du témoin et celui du malade supérieure à 3 minutes au tube contenant la plus grande quantité d'he parine) et surtout un taux du facteur X au-dessous de 10 %. Les valeurs des temps de Quick et du thrombotest étaient trouvées très basses.

Au cours de cette période nous avons pourtant observé des valeurs très basses de ces derniers tests sans manifestations hémorragiques concomittantes. Dans tous ces cas le taux du facteur X était supérieur à 10 et le test de tolérance à l'héparine dans les limites de sécurité (différence entre le temps du témoin et celui du malade inférieure à 3 minutes (fig 2))

Discussion

Il ressort des résultats obtenus par l' pplication des 4 méthodes combinées les faits suivants

) le T.T.H. évolue parallèlement aux fluctuations du facteur X tant u début d traitement qu au cours d un traitement prolongé (fig 3)

Le schéma suivant résume les apports de ces deux tests.

Taux du facteur X > 30 T.T.H. normal ou hypercoagulable

Taux du facteur X < 30 T.T.H. hypocoagulable.

Ce schéma présente toutefois certaines variations d un sujet à l autre Chez certains, le T.T.H. devient hypocoagulable lorsque le taux du facteur X atteint des chiffres inférieurs à 30 et chez d autres, lorsque le taux du facteur X tombe au-dessous de 20 seulement. Ce sont ces malades qui sont le plus menacés d'hémorragie au cours d un traitement nticoagulant.

b) Le temps de Quick présente des cas d'asynchronisme avec le T.T.H qui sont de deux ordres (fig 4)

On observe un allongement très marqué du temps de Quick et un T.T.H. hypercoagulable surtout u début d un traitement, en particulier dans les 3—4 premiers jours. Ceci expliquerait par le fait de la chute en flèche de la proconvertine dès le début du traitement qui influence le temps de Quick. Ce serait une erreur de diminuer la dose de l'anticoagulant, par crainte d'hémorragie, si, en plus, le taux du facteur X est élevé.

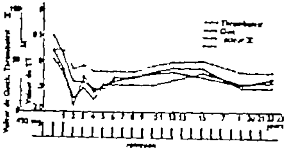


Fig. 1. Parallélisme des valeurs d. T.T.H. et du facteur X et valeurs du thrombotest plus basses que celles du temps de Quick.

Matériel

Ier groupe de 20 malades soumis déjà au traitement anticoagulant par les antivitamines K depuis au moins quinze jours. Le contrôle a été effectué une seule fois pour chaque malade sauf dans quelques cas où celui-ci a été répété à des intervalles très espacés.

Parmi ces 20 cas, 8 concernaient des malades présentant une insuffisance coronarienne, 6 cas d infarctus du myocarde, 3 de thrombophlébite, 1 cas de thrombose cérébrale, 2 de cœur pulmonaire.

2ème groupe de 18 malades contrôlés dès l institution du traitement anticoagulant et suivis pendant une période allant de 15 à 30 jours depuis le début du traitement. Parmi les 18 cas, 9 concernaient des malades présentant un infarctus du myocarde, 3 cas d insuffisance coronarienne, 1 cas d embolie pulmonaire, 1 thrombophlébite, 4 thromboses cérébrales.

Anticoagulants utilisés

Tromexan 19 cas, Sintrom 10 cas, Soluthrombine (phénylindandione) 8 cas. En cours de traitement dans 1 cas, le Sintrom a été remplacé par le Tromexan.

Résultats

Dans le Ier groupe les quatre tests exécutés se trouvent en harmonie complète 20 fois sur 31 mesures.

Le temps de Quick se trouve en désaccord avec les trois autres tests 11 fois sur 31.

Le thrombotest a été trouvé 5 fois en désaccord avec les tests de tolérance à l héparine et le taux du facteur X.

Les valeurs du thrombotest ont été trouvées en général plus basses que celles du temps de Quick.

Le test à l héparine et le taux du facteur Stuart (facteur X) ont été trouvés deux fois en désaccord.

Dans deux cas, parmi ceux de ce groupe qui ont présenté des manifestations hémorragiques plus ou moins sévères, le contrôle du sang de ces malades a montré: dans le Ier cas, le test de tolérance à l héparine indiquait un sang très hypocoagulable (M. 20′ 25″ — T. 12′ 30″), le temps de Quick était à 2 %, le facteur X à 4,5 % et le thrombotest à 4 %.

Dans le deuxième cas les valeurs du temps de Quick et du thrombotest se trouvaient dans les limites dites thérapeutiques, seul le facteur Stuart était dans une zone dangereuse à 11 %.

Dans le deuxième groupe chaque malade a été suivi dès le début du traitement anticoagulant et ensuite pendant une période de 15 à 30 jours. Dans presque tous les cas, on note dès le deuxième jour du traitement une chute verticale du temps de Quick et de celui du thrombotest alors que le test à l héparine révèle le même jour une hypocoagulabilité modérée. (Exemple: Temps de Quick 7,5 %, thrombotest 13, test de tolérance à l'héparine 13′/11′ 30″ (fig. N. 1)). Au cours de la première semaine du traitement, nous n avons jamais observé d incident hémorragique tant que le test de tolérance à l héparine ne montrait pas une hypocoagulabilité excessive et que le taux du facteur Stuart était au-dessus de 15 %.

Au cours de la deuxième semaine de traitement, période à laquelle s observent plus volontiers les manifestations hémorragiques, nous en avons noté dans 6 cas. Le test de tolérance à l héparine montrait alors une nette hypocoagulabilité (diffé-

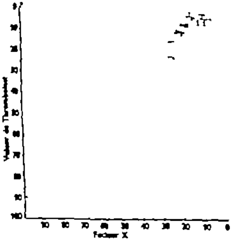


Fig. 7 Comparaison entre les valeurs du facteur X et du thrombotest. Dans une proportion notable les valeurs du thrombotest suivent de près celles du facteur X.

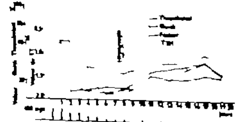


Fig. 8. Au 8ème jour d'un traitement par Tromexan (75 mg) seul le facteur X explique l'incident hémorragique.

d) Le facteur Stuart d'après notre étude, constitue le principal indicateur d une hémorragie imminente lorsque le taux de celui-ci descend au-dessous de 10 %, bien qu'il apparaîtrait que bien d'autres facteurs ou mécanismes non détectés par nos méthodes joueraient un rôle pour l'apparition de ces hémorragies (Poller)

Dans ce cas, comme le préconise d ailleurs Koller il faut diminuer la dose du médicament quel que soit le résultat des autres tests (fig 8)



Conclusions

Il ressort de notre expérience sur la place qu occupe le thrombotest dans l'arsenal des méthodes de contrôle du traitement anticoagulant par les antivitamines K, que cette méthode présente sur les autres tests, en particulier sur le temps de Quick classique, les avantages suivants

1) Théoriquement, le thrombotest contrôle la thromboplastine tissulaire et plasmatique tandis que le temps de Quick se limite à la thromboplastine endogène.

Le fait que sur un sang d'hémophile B, déficient en facteur IX le thrombotest donne des valeurs normales, ne diminue point sa sensibilité en cas de diminution de ce facteur lorsque cette diminution s accompagne parallèlement de la chute des autres facteurs détectés par ce test.

2) La méthode est facile à exécuter et peut se faire même sur sang capillaire.

3) Elle est bien plus sensible que le temps de Quick puisque pour des taux de 100—10 % les temps correspondants varient de 40—200 secondes et la marge des temps thérapeutiques efficaces est nettement plus large allant de 70—120 secondes.

4) La courbe obtenue par les valeurs du réactif sur les dilutions progressives du plasma est très stable. L emploi d un réactif standard élimine toutes les vicissitudes des différentes thromboplastines préparées par les différents laboratoires Les résultats obtenus peuvent être comparés entr'eux sans avoir à tenir compte, comme cela arrive souvent, du laboratoire qui a donné ces valeurs.

5) Enfin, après un traitement prolongé, il suit en général de plus près les fluctuations du facteur Stuart, qui, comme

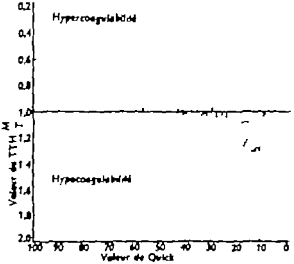


Fig 4 Comparaison entre les valeurs de Quick et du T T H Les valeurs du temps d Quick inférieures à 40 correspondent dans une grande proportion aux valeurs du T T H exprimant l'hypocoagulabilité sanguine globale Les cas de discordance sont pourtant plus nombreux que dans le cas du thrombotest (fig 6)

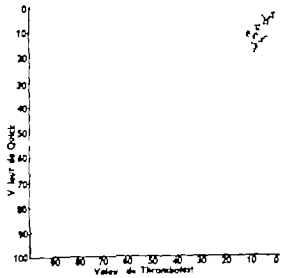


Fig 5 Comparaison entre les valeurs du temps de Quick et du thrombotest On peut noter que les valeurs du thrombotest sont, en général, plus basses que celles du temps de Quick.

Il est habituel dans les premiers jours d observer un taux de Quick au-dessous de 10 % sans que cela soit dangereux pour le malade

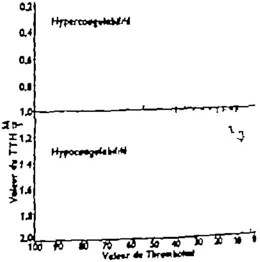


Fig 6. Comparaison entre les valeurs du thrombotest et du T.T.H. Les valeurs du thrombotest inférieures à 40 % correspondent, dans la majorité des cas, aux valeurs du T T H qui expriment l'hypocoagulabilité sanguine globale
Il exist pourtant des cas de discordance mais ils sont beaucoup plus rares que dans la comparaison avec le temps de Quick (fig 4).

Lors d un traitement prolongé, on observe d habitude, un léger allongement du temps de Quick avec T T H. hypocoagulable ce phénomène peut apparaître pour la première fois, bien longtemps après le début du traitement. Il n est pas nécessaire alors d augmenter la dose du médicament d autant plus que le facteur \ est très bas. Le danger d'hémorragie est réel.

c) Les valeurs obtenues par le thrombotest concordent en général au début du traitement avec celles données par le temps de Quick avec toutefois des valeurs plus basses que celui-ci et ce serait de même commettre la même erreur de diminuer la dose de l anticoagulant par crainte d hémorragie (fig 5) Mais, par la suite lors d un traitement prolongé le thrombotest suit en général de plus près les fluctuations du facteur \ ce qui permet de contrôler le traitement anticoagulant avec moins de risques qu avec celui du temps de Quick (fig 6—7)

Summary

The authors have studied the fluctuations of the thrombotest, Quick's time test using human thromboplastin, the heparin tolerance test, the Stewart factor (X) and their interrelationship in 33 cases submitted to anticoagulant treatment with anti-vitamin K on account of thromboembolic episodes.

It was observed that the changes in the heparin tolerance test followed closely those of factor X. Consequently, in the event of asynchronism between the Quick's time test or thrombotest and the heparin tolerance test, there is only factor X to depend upon.

The authors conclude that the thrombotest constitutes an excellent method of controlling anticoagulant treatment, superior to all previous tests and especially the Quick's time test, for examining a very large number of cases.

A schematic presentation is given of the mode of the anticoagulant treatment and its control. The doses of anticoagulant drugs used in Greece are notably lower than those administered in other countries.

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nous l'avons dit plus haut, constitue le principal guide pour la prévention des hémorragies.

Le thrombotest présente toutefois *quelques inconvénients* pour le contrôle d'un traitement anticoagulant.

1) Pour obtenir une hypocoagulabilité réelle, comme le montre d'ailleurs le test de tolérance à l'héparine, il faut que les 4 ou 5 premiers jours du traitement par les antivitamines K le taux du thrombotest descende très bas, quelquefois même au-dessous de 10 %, comme d'ailleurs il arrive avec le temps de Quick.

2) Son principal inconvénient réside dans le fait que le thrombotest ne constitue pas un test de coagulabilité globale comme le sont le T.T.H. et la thrombélastographie.

Son prix reste encore élevé malgré toutes les tentatives de diminution de la quantité de réactif utilisée et les efforts réels fournis par la maison qui le fabrique. Il n'y a pas de doute que le thrombotest, comme l'a affirmé maintes fois Owren, n'est point destiné à des laboratoires spécialisés à la thérapeutique anticoagulante, mais à ceux dont les moyens sont limités. A notre avis ce test vient combler les lacunes importantes des autres tests utilisés en grande série, en particulier le temps de Quick.

Pour terminer, nous exposons d'une façon schématique les modalités d'application du traitement anticoagulant par les antivitamines K, le mode de surveillance de ce traitement tel que nous le pratiquons.

Au début du traitement les doses d'attaque sont importantes afin que le résultat soit obtenu d'une façon rapide et efficace.

Dans notre pays, en Grèce, les doses habituellement indiquées sur le prospectus des différents médicaments nous semblent exagérées et, dans de nombreux cas, dangereuses pour le malade. En effet, nous avons maintes fois constaté que les doses que nous employons en Grèce sont beaucoup plus faibles que celles utilisées dans les autres pays. C'est un fait difficile à expliquer. Il se peut qu'un mauvais fonctionnement hépatique ou des raisons climatiques jouent un certain rôle dans la production des facteurs de la coagulation.

Pour les anticoagulants, employés couramment, les doses d'attaque sont les suivantes: Tromexan 600 mg, Sintrom 6—8 mg, Soluthrombine 200—300 mg et Dicandin 10—15 mg.

La diminution progressive des doses commence dès le troisième jour du traitement jusqu'au dixième jour environ. Dès lors nous maintenons une dose d'entretien régulière qui dépendra de l'évolution chronique et des résultats du laboratoire.

Au début du traitement, le 1er et le 2ème jour, nous mesurons le temps de Quick ou le thrombotest et faisons un T.T.H. En cas d'asynchronisme, nous nous basons sur les résultats de ce dernier.

Entre le 3ème et le 10ème jour: T.T.H. tous les deux jours et détermination du facteur X afin d'éliminer le danger d'hémorragie.

Traitement prolongé: Temps de Quick ou thrombotest tous les 15 jours durant les 3 premiers mois, puis une fois par mois. Le T.T.H. se fera d'une façon espacée afin de contrôler l'efficacité du traitement. Dans les cas de discordance entre le temps de Quick, thrombotest et le T.T.H. on se basera surtout sur les résultats de ce dernier en s'aidant parfois de la détermination du facteur X.

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Serum Proteins and Glucoproteins in Normal Schoolchildren

By

L. E. Böttiger and G. Sterky

The greatly increased interest in the protein-bound carbohydrates of serum is reflected by the large number of publications on the subject during recent years. Most of them deal with adult materials and we have not been able to find any complete study of serum glucoproteins during childhood and adolescence. This is a report of a study on the sera of more than 100 children, aged 7—20 years, in which analyses were made of protein-bound hexoses, hexosamines and sialic acids as well as of the total protein and electrophoretic protein distribution. Corresponding values for adult men were published in 1960 (1) and values for new born children and infants in 1962 (2).

Material and methods

The material consists of the matched controls of all diabetic schoolchildren in Stockholm, in all 121 children, aged 7—20. The mode of selection is described in connection with study on the diabetic material (7). Only children in good health and with a normal ESR (≤ 10 mm/hr Westergren method) at the time of blood sampling were included in the final material. After exclusion of 16 children with elevated ESR, the final material comprised 105 children, 50 boys and 55 girls, evenly distributed among the age groups (cf. table 1).

Blood samples were taken at the beginning of the school term in the fall of 1960. Serum was separated within a few hours and stored in the frozen state until analyzed.

Total protein was determined by a biuret method.

Paper electrophoresis was carried out in a barbital buffer of pH 8.6 and ionic strength 0.1. The strips were stained with bromphenol blue, cut, the dye eluted and the concentration of the dye measured spectrophotometrically.

Hexoses were determined with an anthrone reagent, *hexosamines* by the Elson-Morgan procedure and *sialic acids* with the resorcinol-Cu reagent of Svennerholm. The methods used have been described previously (1).

Results

The compounds analyzed showed a normal distribution. As there was no difference in the values between the

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Table III The influence of slightly elevated ESR on the glucoprotein values

Mean values ± standard deviation.

	No.	Protein-bound carbohydrates (mg/100 ml)			
		Hexoses	Hexosamines	Sialic acids	Total
Children with elevated ESR (11—20 mm/hr)	16	122 ± 12.5	96 ± 8.1	67 ± 7.8	285 ± 25.8
Normal children (ESR ≦ 10 mm/hr)	103	107 ± 8.7	86 ± 7.4	58 ± 4.4	252 ± 18.3
Significance of difference (t-test)		*			

that with age there is a probable increase for the sum of the glucoproteins (hexoses + hexosamines + sialic acids) — regression equation $Y = 1.35x + 234$ $S_y = 0.60$ $0.01 < p < 0.05$ but that the increases in the single components do not reach statistical significance for hexoses and sialic acids. The molecular ratios between the different compounds were the same here as in the material published previously (1) i.e. 3.1 : 2.5 : 1 (hexoses : hexosamines : sialic acids).

The total protein and the electrophoretic distribution is given in table II. Total protein values show a slow increase with age, likewise the albumin and gamma globulin fractions. Regression analysis however shows that there is no statistically significant increase in the albumin values, that there is only a probable increase in the total protein values and a definite increase only in the gamma globulins.

The differences between the lowest and the highest values of the different fractions of proteins and glucoproteins were, however comparatively small and the sample standard deviation for the entire material and from regression were of the same order of magnitude. It was therefore concluded permissible to calculate mean values etc. for the entire material. These values are found at the bottom of the tables.

Discussion

As stated in the introduction we do not know of any published material that has been analyzed for the different glucoproteins as in our study. Figures reported for serum glucoprotein hexoses and "glucosamine" (5 6) in a small group of children agree well with those reported here.

When reporting normal glucoprotein values, it is of utmost importance to take great care in the selection of the material. Even a small increase in ESR is followed by a marked and significant increase in the glucoprotein values. Table III shows the mean values for the 16 children excluded because of elevated ESR. Twelve of these had an ESR between 11 and 15 mm/hr and only 4 had values between 16 and 20 mm/hr. There was no difference between these groups but the very marked difference between the children with an ESR ≦ 10 and those with values between 11 and 20 mm/hr is clearly demonstrated.

Regarding total protein and the electrophoretic pattern there exist several

Table I. Values for protein-bound carbohydrates in different age groups

Mean values ± standard error of the mean and (below) standard deviation are given.

Age group	No.	Protein-bound carbohydrates (mg/100 ml)			
		Hexoses	Hexosamines	Sialic acids	Total
7−10	23	106 ± 1.9 8.9	84 ± 1.5 7.1	57 ± 0.8 3.9	247 ± 3.8 18.5
11−1	18	107 ± 2.1 9.1	86 ± 1.9 8.2	58 ± 1.2 4.9	251 ± 4.7 19.8
13−14	22	107 ± 1.7 7.9	85 ± 1.7 7.9	57 ± 0.8 3.7	249 ± 3.5 16.3
15−16	25	106 ± 1.5 7.3	86 ± 1.3 6.3	57 ± 0.8 4.2	250 ± 3.2 15.9
17−20	17	112 ± 2.4 9.7	91 ± 1.5 6.1	60 ± 1.3 5.2	264 ± 4.6 18.9
Total 7−20	105	107 ± 0.9 8.7	86 ± 0.7 7.4	58 ± 0.4 4.4	252 ± 1.8 18.3

Table II. Total protein and electrophoretic distribution in different age groups

Mean values ± standard error of the mean and (below) standard deviation are given.

Age group	No.	Total protein g/100 ml	Electrophoretic fractions				
			Albumin	Globulins			
				α	α_2	β	γ
7−10	19	7.06 ± 0.08 0.34	4.61 ± 0.11 0.46	0.29 ± 0.02 0.09	0.54 ± 0.02 0.08	0.59 ± 0.02 0.07	1.04 ± 0.05 0.23
11−12	15	7.01 ± 0.06 0.[illegible]4	4.62 ± 0.07 0.28	0.27 ± 0.01 0.04	0.51 ± 0.01 0.06	0.60 ± 0.02 0.07	1.01 ± 0.05 0.19
13−14	21	7.09 ± 0.10 0.44	4.33 ± 0.06 0.28	0.30 ± 0.01 0.04	0.55 ± 0.01 0.06	0.63 ± 0.01 0.0[illegible]	1.10 ± 0.05 0.23
15−16	25	7.13 ± 0.07 0.34	4.71 ± 0.08 0.39	0.28 ± 0.01 0.05	0.52 ± 0.01 0.06	0.61 ± 0.02 0.08	1.04 ± 0.03 0.15
17−20	16	7.39 ± 0.11 0.44	4.78 ± 0.10 0.39	0.31 ± 0.01 0.04	0.53 ± 0.01 0.04	0.63 ± 0.02 0.08	1.15 ± 0.04 0.15
Total 7−20	96	7.15 ± 0.04 0.39	4.65 ± 0.04 0.38	0.29 ± 0.01 0.06	0.53 ± 0.01 0.06	0.61 ± 0.01 0.07	1.07 ± 0.02 0.20

sexes the only division of the material is into age groups. The results of the glucoprotein analyses are illustrated in table I.

The glucoprotein values for the youngest children are within the normal range for adults (1) although the mean values are significantly lower. Glucoprotein values tend to increase slowly during the period of growth, an increase of approximately 6 %. Regression analysis shows

Table III The influence of slightly elevated ESR on the glucoprotein values

Mean values ± standard deviation.

	No.	Protein-bound carbohydrates (mg/100 ml)			
		Hexoses	Hexosamines	Sialic acids	Total
Children with elevated ESR (11—20 mm/hr)	16	122 ± 12.5	96 ± 8.1	67 ± 7.8	285 ± 25.8
Normal children (ESR ≦ 10 mm/hr)	105	107 ± 8.7	86 ± 7.4	58 ± 4.4	252 ± 18.5
Significance of difference (t-test)					

that with age there is a probable increase for the sum of the glucoproteins (hexoses + hexosamines + sialic acids) — regression equation $Y = 1.35x + 234$ $S_b = 0.60$ $0.01 < p < 0.05$ but that the increases in the single components do not reach statistical significance for hexoses and sialic acids. The molecular ratios between th different compounds were the same here as in the material published previously (1) i.e. 3.1 2.5 1 (hexoses hexosamines sialic acids)

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The differences between the lowest and the highest values of the different fractions of proteins and glucoproteins were however comparatively small and the sample standard deviation for the entire material and from regression were of the same order of magnitude. It was therefore concluded permissible to calculate mean values etc. for the entire material. Those values are found at the bottom of the tables.

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As stated in the introduction we do not know of any published material that has been analyzed for the different glucoproteins as in our study Figure reported for serum glucoprotein hexoses" and glucosamine (5,6) in a small group of children agree well with those reported here.

When reporting normal glucoprotein values, it is of utmost importance to take great care in the selection of the material. Even a small increase in ESR is followed by a marked and significant increase in the glucoprotein values. Table III shows the mean values for the 16 children excluded because of elevated ESR. Twelve of these had an ESR between 11 and 15 mm/hr and only 4 had values between 16 and 20 mm/hr There was no difference between these groups but the very marked difference between th children with an ESR ≦ 10 and those with values between 11 and 20 mm/hr is clearly demonstrated.

Regarding total protein and the electrophoretic pattern there exist several

previous studies on children (see for example Oberman et al. 1956 (4) or Gyllenswärd and Josephson 1957 (3) both including a summary of previous results). As found by these and other authors, values as early as at the age of 7—10 years are within the normal range for adults but there is a small increase in total protein, albumin and gamma globulins during the periods of growth. There is no evident difference in the small intermediate fractions of alpha and beta globulins.

The results show that even if the values for children are within the normal adult range, their mean are nevertheless significantly lower. This implies that special normal values for children such as those reported here for protein and glucoproteins, should be used as a basis for studies on pathological pediatric materials.

Summary

The total protein, protein electrophoretic pattern and protein bound carbohydrates of serum (hexoses, hexosamine and sialic acids) were analyzed in sera from 105 normal children aged 7—20 years.

Special emphasis is laid on the fact that even a very small increase in ESR will give a considerable rise in serum glucoprotein values.

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Serum Proteins and Glucoproteins in Diabetic Schoolchildren

By

G. Sterky and L. E. Böttiger

Many different ways of trying to elucidate the pathogenesis and development of diabetic angiopathy have been reported. Among other factors the serum proteins and glucoproteins have been studied in diabetic patients with and without various stages and types of vascular damage. Most studies have concerned themselves with adult materials including only a few young patients and there is sparse information about the concentration of protein and protein-bound carbohydrates in diabetic children (19, 20, 37).

This investigation was undertaken to determine the distribution of these compounds in children and adolescents with juvenile diabetes. Because of reports on an elevation of the glucoproteins in patients with diabetes mellitus, especially in the presence of vascular disease, we have considered it of importance to study a material before the appearance of such changes. The concentration of protein-bound hexoses, hexosamines and sialic acids in whole serum are given in this paper together with the electrophoretic protein pattern.

Submitted for publication March 1, 1962.

Material and clinical methods

All diabetic children attending the schools in Stockholm were registered. These 165 children (75 ♂, 90 ♀) were invited to take part in an investigation carried out during the autumn of 1960 and the spring of 1961. For different reasons, unwillingness to take part and acute infections, only 134 (81.2 %) are included in the investigation on glucoproteins. On the day of sampling (at the beginning of the school term in Sept. 1960), the ESR (Westergren method) was determined and urinanalyses for protein, sugar and acetone made. The fasting blood sugar level was determined in 45 diabetic cases. Throats were inspected at the time of sampling and during the period of observation of the study each child was given a careful clinical examination by one of the authors (G. S.). Children with signs of major diseases other than diabetes were excluded.

All the diabetics were under regular medical supervision and all were insulin-treated with the exception of two who were at the time in a state of remission. The duration of diabetes in the material varies from 8 months to 18 years (mean 5.8 years) but only 13 had a duration of more than 10 years. Onset in all cases but 3 took place before the age of 16 and invariably resulted in hospitalization. Later supervision

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previous studies on children (see for example Oberman et al 1956 (4) or Gyllenswärd and Josephson 1957 (3) both including a summary of previous results) As found by these and other authors values as early as at the age of 7—10 years are within the normal range for adults but there is a small increase in total protein albumin and gamma globulins during the periods of growth. There is no evident difference in the small intermediate fractions of alpha and beta globulins.

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Summary

The total protein, protein electrophoretic pattern and protein-bound carbohydrates of serum (hexoses, hexosamines and sialic acids) were analyzed in sera from 105 normal children aged 7—20 years.

Special emphasis is laid on the fact that even a very small increase in ESR will give a considerable rise in serum glucoprotein values.

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Table III Protein-bound carbohydrates in different age groups of the final material for boys and girls
Mean values and standard error of the mean and (below) standard deviation are given.

Age group (years)	Protein-bound carbohydrates (mg/100 ml)				
	No.	Hexoses	Hexosamines	Sialic acids	Total
			— Boys —		
7–10	8	117 ± 3.7 16.2	93 ± 3.6 10.3	63 ± 1.8 4.0	273 ± 11.0 31.1
11–12	7	117 ± 5.8 15.5	89 ± 2.1 5.5	63 ± 1.4 3.6	269 ± 8.7 23.0
13–14	11	112 ± 2.7 8.8	85 ± 1.9 6.2	59 ± 1.3 4.2	255 ± 5.3 17.5
15–16	3	117 ± 2.7 1.5	91 ± 2.3 4.0	60 ± 0.6 1.0	268 ± 3.0 5.2
17–20	6	126 ± 5.5 13.4	92 ± 2.0 5.0	62 ± 1.5 3.8	279 ± 7.6 18.6
			— Girls —		
7–10	10	113 ± 3.0 9.3	88 ± 2.6 8.1	62 ± 1.8 5.6	263 ± 7.1 22.4
11–12	6	119 ± 3.8 9.3	86 ± 4.0 9.7	59 ± 2.6 6.4	264 ± 10.1 24.8
13–14	10	115 ± 2.6 8.3	85 ± 3.1 9.7	60 ± 1.5 4.6	260 ± 5.5 17.5
15–16	8	128 ± 4.0 11.2	90 ± 3.7 10.6	67 ± 2.3 6.6	285 ± 8.2 23.2
17–20	2	123	98	68	288

ent deviation from the normal distribution was found in the histograms of the compounds analyzed. The distribution of the protein-bound carbohydrates with in the various age groups is presented in table II. The influence of elevated ESR on the concentration of glucoproteins motivated the exclusion of 11 cases of elevated ESR, even those slightly raised (cf. Böttiger & Sterky (7) and discussion below). With regard to the aim of this study and the presumed influence of diabetic retinopathy and nephropathy on the glucoprotein concentrations in serum, the 20 cases with clinical manifestations of angiopathy were also excluded. The distribution of the various protein-bound carbohydrates in the thus reduced, final material is given in table III. Total protein-bound carbohydrates (hexoses + hexosamines + sialic acids) for the various age groups and the individual values for the angiopathy cases are shown in fig 1.

The individual values for hexoses, hexosamines and sialic acids were found to run a parallel course. No change with age could be demonstrated for the total values (hexoses + hexosamines + sialic acids) by means of linear regression

Table I. Composition of the material

	Boys	Girls	Total
No. examined	62	72	134
Cause of rejection			
ESR not determined	3	5	
Angiopathy not evaluated	1	0	
ESR above 21 mm/hr	1	3	
Other reasons	2	2	
	7	10	17
No. accepted	55	62	117
Cases with ESR 11—20 mm/hr	10	20	30
Cases with angiopathy	12	8	20
Cases with normal ESR and no angiopathy (final material)	35	36	71

Two cases in each group had ESR 11—20 mm/hr

was carried out by various doctors at different centres who held differing opinions concerning treatment, especially as regards the intensity of dietary prescription. All children were examined by an ophthalmologist for the evaluation of retinopathy. Nephropathy was diagnosed in cases where there was sporadic or permanent proteinuria. At the diabetic clinic of Crown Princess Lovisa's Children's Hospital continual supervision was carried out on about 45 % of this material. The principles of treatment at this hospital and the criteria for grading the different stages of angiopathy have been reported on by Larsson and Sterky (21). The degree of diabetic control has been evaluated on the basis of in- and out patient records and a good personal knowledge of the daily lives of the patients (34).

Biochemical methods

All blood samples were withdrawn in the morning in the fasting state before the children had received their insulin. Serum was separated within 1—2 hours and stored in the frozen state until analyzed. *Total protein* was determined by a micro-biuret procedure. *Paper electrophoresis* was performed in a barbital buffer at pH 8.6 with subsequent staining and elution of the dye. *Hexoses* were analyzed with an anthrone reagent, *hexosamines* by the Elson

Table II. Protein-bound carbohydrates in different age groups of the total material for boys and girls

Mean values and, for the total values, standard error of the mean and (below) standard deviation are given.

Age group (years)	Protein-bound carbohydrates (mg/100 ml)				
	No.	Hexoses	Hexosamines	Sialic acids	Total
— Boys —					
7—10	11	116	92	63	270 ± 8.3 27.7
11—12	11	118	89	63	269 ± 5.9 19.7
13—14	16	115	88	60	264 ± 5.0 20.0
15—16	8	117	89	59	265 ± 2.6 7.5
17—20	9	128	91	62	282 ± 6.3 19.0
— Girls —					
7—10	15	116	90	63	268 ± 5.6 21.6
11—12	9	124	89	62	274 ± 9.8 29.4
13—14	14	120	87	63	270 ± 6.4 23.8
15—16	16	134	93	68	295 ± 9.1 36.2
17—20	8	123	95	64	282 ± 5.8 16.5

Morgan procedure, and *sialic acids* with Svennerholm's resorcinol-Cu reagent (4). *Zone electrophoresis* in polyvinyl chloride was performed as described by Böttiger and Carlson (3).

Results

The reasons for rejection of certain cases and the composition of the different materials are given in table I. No appar

Table IV Total protein and electrophoretic protein distribution among diabetic and non-diabetic schoolchildren

Mean values and standard error of the mean and (below) standard deviation are given.

Material	No.	Total protein (g/100 ml)	Electrophoretic fractions (g/100 ml)				
			Albumin	Globulins			
				α_1	α_2	β	γ
Diabetics.	68	6.96 ± 0.06	4.44 ± 0.06 0.49	0.30 ± 0.01 0.06	0.58 ± 0.01 0.08	0.60 ± 0.01 0.08	1.04 ± 0.02 0.18
Non-diabetics	96	7.15 ± 0.04	4.63 ± 0.04 0.38	0.29 ± 0.01 0.06	0.53 ± 0.01 0.06	0.61 ± 0.01 0.07	1.07 ± 0.02 0.20
Significance of difference (t-test)		*		–		–	–

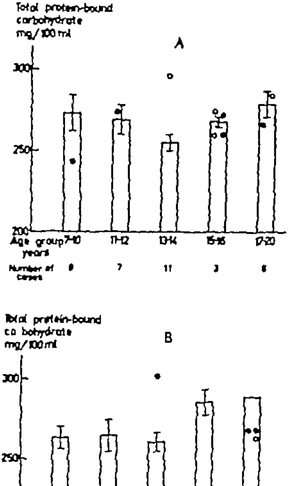
6—10 and more than 11 years, no correlation was found between the various glucoproteins and the duration. The *diabetic control* was significantly worse among girls than among boys (35) but the degree of control had no influence upon the concentration of the various protein-bound carbohydrates, either among boys or girls. Nor could any correlation be shown between the glucoprotein level and the glycosuria of the preceding night or the fasting blood sugar level at the time of sampling.

In 3 cases, not included in this series, of advanced diabetic angiopathy (proliferative retinopathy, severe vascular calcification and permanent proteinuria) some electrophoresis of the glucoproteins was carried out. No pathological component was observed in the various electrophoretic fractions and the ratios between the glucoproteins included in all fractions were normal.

The results obtained by paper electrophoresis of serum proteins are given in table IV. Total protein and albumin showed a certain, though not significant increase with age as in the control material. No sex difference was found. When comparing the results in the final diabetic material with those of the matched controls a significant increase in α_2-globulin and a decrease in albumin and total protein was found.

Discussion

The values for the different protein-bound carbohydrates reported here are somewhat higher than the values for non-diabetic children in the same age groups (7). However, a statistically significant difference was found only in the 13—16 age group of the girls. The serum levels of hexoses and hexosamines in our group of uncomplicated diabetes are lower than those given in other studies on diabetic patients. Sialic acid values are only given by a few authors (16, 32). There seem to be no studies which can be directly compared with ours as different biochemical methods and different criteria for the selection of the materials have been used. Korc *et al.* (19) however reported on a study on protein-bound hexoses in 50 diabetic children who were said to suffer no complications. Their mean value of 131.1 mg/100 ml is higher than in any of our age groups.



Figs. 1 A (boys) and B (girls) Total protein-bound carbohydrates (hexoses + hexosamines + sialic acids) in different age groups of the final material. Each column represents the mean value of the age group with the standard error indicated. Each o represents one case of angiopathy

analysis. As appears from fig 1 however a change seems to take place at puberty with the highest values occurring after puberty Between the age groups 7—16 and 17—20 in the boys, however there is no significant difference (t test) The girls, on the other hand show a statistically significant difference ($p < 0.01$) both for the total values as well as for hexoses between the age groups 7—14 and 15—20

The mean values for the various protein bound carbohydrates in the final material were compared with corresponding values in a matched control material of non-diabetics (7) with attention paid to sex and age. The mean values are higher throughout the diabetic material, but a statistically significant difference could be shown only for the hexoses and the total values in the 15—16 age group of the girls ($p < 0.001$)

The molecular ratios were calculated at different concentrations of sialic acid and were found to be identical with those earlier reported (4 7)

Elevated ESR (11—20 mm) appeared more frequently in diabetic girls (statistically insignificant) just as frequently in all age groups and did not appear more frequently in angiopathy cases than in the others. Nor could the duration of diabetes, age at onset or degree of diabetic control be connected with the findings of increased ESR. The sera of 16 patients with an initial elevated ESR were examined at later sampling and the rate was then found to have risen in 1 patient, remained unchanged in 3 and decreased in 12

In order to attempt to decide whether factors other than increasing age influence the level of glucoproteins among juvenile diabetics, the final material was also investigated with regard to age at onset duration of diabetes and degree of diabetic control. The mean values for the total protein-bound carbohydrates in the different groups were compared with *age at onset* i e. 0—5 6—10 or more than 11 years. No statistical difference was found. When dividing the final material into groups with a *duration of diabetes* of 0—5

ESR first observed is temporary and caused by non-diabetic factors, possibly by an increased susceptibility to infection in the juvenile diabetics (for discussion see Sterky (35))

Besides changes due to age, infection or vascular damage, many other factors have been investigated to explain the observed increase in glucoproteins among diabetics. The age at onset of diabetes is said to be of certain significance by some authors (12) but the results of this study and also the observations of others (19) in purely juvenile materials do not support the statement. Nor has the duration any influence on the glucoprotein level in materials in which all cases with various types of complication are excluded (26) this being in agreement with our findings. The degree of diabetic supervision has been investigated by many authors with varying results (17 25) Ours do not indicate that this factor is of any importance.

The serum proteins in diabetes have been studied a number of times. There are, however only few results published on materials consisting of diabetic children. However Traisman et al. (37) studying material similar to ours, found a β-globulin increase and γ-globulin decrease whereas Korp and Formanek (20) found tendency towards a decrease in albumin and an increase in α_1- and α_2-globulins. On comparison with non-diabetics the most common finding has been an γ-globulin increase and an albumin decrease (12, 17 21 27) Our results are in agreement with those of Schertenleib and Tuller (29) who suggested that an increase in α_2-globulin may be found before angiopathy can be diagnosed clinically. As with the glucoproteins, it is of the utmost importance when judging the protein pattern that the patients are quite free from intercurrent infection.

The internal relationship between the various protein-bound carbohydrates is the same for all diabetics in this investigation regardless of the type of selection, the ratio does not differ in diabetics compared with non-diabetics (7) and no abnormal protein component seems to appear among the diabetics.

Whether the augmented value of any of the protein and/or glucoprotein compounds in an individual diabetic patient implies greater risk of premature vascular injury cannot yet be decided. Factors of prognostic value are much needed as we have no reliable criteria for judging the degree of severity of juvenile diabetes.

Summary

The protein electrophoretic patterns and serum protein bound carbohydrates (hexoses hexosamines and sialic acids) of 134 diabetic schoolchildren aged 7—20 years are analyzed.

The values for protein bound carbohydrates tend to be somewhat higher in the diabetics than in a corresponding material of metabolically healthy children, although the differences with a few exceptions do not reach statistical significance.

No correlation is found between the levels of protein-bound carbohydrates and the age of onset, the duration or the degree of control of the diabetes.

The protein patterns of the diabetics show a significantly elevated α_2-globulin level and a decreased albumin and *total* protein content.

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In the age groups studied there is no clear continuous increase in glucoprotein values with age. During puberty, however, a gradual increase does occur, most apparent in the girls. As these years seem to have special importance as regards the long-term prognosis of juvenile diabetes (15, 21) our findings are of interest. The increase may be in agreement with the findings of Sirek et al. who in studies on the offspring of diabetic mothers (32) and alloxan diabetic rats (33) stated that the elevation is most pronounced at the time of rapid growth of tissue.

In our material there are relatively few patients with angiopathy, and these have suffered changes of moderate severity alone. It is therefore not possible to reach any conclusion as to the relationship between the serum glucoprotein level and the vascular findings in long term diabetes. Some of the arterial changes seen in diabetes are undistinguishable from non-diabetic atherosclerosis but other findings seem to point to more specific diabetic vascular damage (for discussion see Larsson and Sterky (21)). The findings of Randerath and Dietzel (28) of a considerably larger amount of acid mucopolysaccharides in the intima of minute muscular blood vessels in diabetics than in non-diabetics are thus of importance (9). If there is a connection between the serum glucoproteins and the mucopolysaccharides of the ground substance (11, 31, 32) it is possible that an elevation of the serum glucoproteins is an indication of the appearance of vascular changes before it is clinically possible to diagnose them.

Many authors have reported elevated protein bound carbohydrates in non-diabetic arterial disease (30), but others have found normal values when strict criteria were applied for the selection of the materials (6). In advanced diabetic nephropathy elevated glucoproteins have been found (1, 3, 14, 25, 36). As regards the level of protein bound carbohydrates in patients with retinal microaneurysms (to which stage of angiopathy most of our patients belong) some authors have found increased values (2, 10, 12, 18, 24), but others glucoproteins as in uncomplicated diabetes (8, 13, 17, 22). Many unknown relationships and points of obscurity remain to be investigated before the serum glucoprotein level can be correlated with diabetic angiopathy.

It is well known that many different factors may cause an increase in the concentration of protein bound carbohydrates. As we have shown in another paper (7), even a slight elevation of the ESR implies an increase in all glucoprotein fractions. This is also the case with our diabetes material, an observation which has been made previously (24). The question quite naturally is whether, and in which case how, the occurrence of a slightly elevated ESR is connected with the diabetes. It has been shown that an elevated ESR occurs frequently in patients with long-duration juvenile diabetes (21, 23). We have not been able to correlate the frequency of elevated ESR with the duration of diabetes, age at onset, degree of diabetic control or occurrence of angiopathy. In order to discover whether an elevated ESR and the concomitant increase in the glucoproteins is the result of any specific metabolic disturbance common to juvenile diabetics, repeated examinations were made of a number of the patients who initially had slightly elevated ESRs. Few of the cases so examined did not exhibit a fall in the ESR and glucoproteins. It thus seems reasonable to assume that the elevation of the

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Changes in the Flora of Urinary Tract Infections during Antibacterial Therapy

By

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Changes in the flora of a urinary tract infection frequently occur during anti microbial therapy Stenderup et al. (1952 (10)) observed this 29 times during 252 treatments Rhoads et al. (1952 (9)) 46 times in 159 patients Gould et al. (1953 (3)) 4 times in 14 patients Garrod et al. (1954 (2)) 68 times in 686 patients. Middleton (1957 (6)) mentions the conversion of a proteus infection to a pyocyaneus infection during nitrofurantoin therapy The bacteria found during or after treatment are alw ys resistant to the antibacterial drug used. Since fresh infection from outside the urinary tract is unlike in most of these cases, the change of flora from one bacterial species to another is poorly understood. It cannot be explained as development of resistance during antibacterial therapy since there is a change of bacterial species.

In our opinion, a mixed infection, consisting of great number of bacteria of one species with only a few bacteria of another species, exists in these cases prior to the commencement of treatment. In the usual routine examination of the urine only the species present in the greatest number will be found, and treatment directed against these organisms will cause their disappearance. However should the other species be insensitive to the drug used, it will be able to multiply freely during treatment. Although in continued observations of numerous urinary tract infections this hypothesis proved its worth, the sequence of events is difficult to demonstrate. In our opinion, the following two case histories provide this evidence. Both concern male patients with a long history of urinary tract infections, and who were able to urinate spontaneously during the course of this study

Methods

The urethral orifice was cleaned with lysol (1/000) and midstream sample of urine was collected and examined within one hour A urinary tract infection was only diagnosed when pyuria was present in the centrifuged urinary sediment and bacteria found in the Gram-stained smear of this sediment. These bacteria were identified with ordinary culture methods. Sensitivity tests were made with paper discs.

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Case reports

Case 1 65-year-old male. Following cholecystectomy on Dec. 7 1953, the patient required an indwelling catheter for 3 months due to urinary retention. Bacteriologic examination of the urine revealed Proteus vulgaris and Streptococcus faecalis. During this period the patient developed a pulmonary infarction, which was found to be infected with resistant hospital staphylococci, sensitive only to chloramphenicol and erythromycin. The resulting staphylococcal pneumonia resolved with chloramphenicol therapy On March 15, 1954 following transurethral resection of the prostate the indwelling catheter was removed. Renal function was normal, radiological examination did not reveal any renal calculi

On March 23, 1954, Gram-stain of the urinary sediment revealed the presence of many Gram-positive diplococci and Gram-positive cocci arranged in clusters. S. faecalis, S aurei sensitive only to nitrofurantoin and erythromycin, and few P vulgaris were cultured.

A blood culture made on April 4 because of rise in the patient's temperature, grew S. aurei with the same resistance pattern as the staphylococci isolated from the urine. The patient was given 2 g erythromycin for only 7 days during his period the temperature became normal. Table I illustrates the course of the urinary infection. S faecales disappeared completely staphylococci only slowly and few Pr vulgaris were still found in the cultures. Since the isolated Pr vulgaris were sensitive to nitrofurantoin in vitro, 400 mg of this drug were administered daily for 8 days. On the second day of treatment, however the urinary sediment contained more Gram-negative rods than before hich, on culture, proved to be Pseudomonas aeruginosa (pyocyanea) Owing to he fact that the Ps. aeruginosa which had been isolated was sensitiv to streptomycin in vitro, the therapy was changed to 2 g streptomycin m. and 4 g supronal (sulphonamide especially recommended for this type of infection at tha time The urine became clear without further evidence of pus cells or bacteria therapy was discontinued after 6 days.

Five days after terminating this treatment P vulgaris and resistant S. aurei reappeared in the urine only to disappear again during treatment with nitrofurantoin. Eventually these organisms were replaced by Ps. aeruginosa (see table I)

On May 6, a rise in the patient's temperature was again associated with a positive blood culture for S aurei, sensitive only to erythromycin. In the course of 6 weeks, the patient received 2 g erythromycin daily and completely recovered from his systemic staphylococcal infection. The urine always contained many pus cells and numerous Ps. aeruginosa.

Finally 2×50 mg polymyxin i. m. and 4×250 mg oxytetracyclin were given daily for 6 days. Ps. aeruginosa disappeared rapidly but after discontinuing treatment the urine contained many S aurei, which were sensitive only to chloramphenicol. Without further treatment, a change in flora was again demonstrated one month later when pyuria exclusively with Ps. aeruginosa was found.

Comments

In our opinion the explanation of the changes in the urinary bacterial flora of this patient is as follows. The prolonged use of an indwelling catheter established an infection with Pr vulgaris, Ps. aeruginosa (pyocyanea) S faecalis and a hospital S. aureus. Since transurethral prostatectomy is only a partial prostatectomy the remnants of the prostate can harbour organisms which are difficult to reach with antibacterial drugs. During subsequent treatments, Pr vulgaris and S. faecalis were probably eliminated but an pparently mixed infection with staphylococci and Ps. aeruginosa remained. However this mixed" infection was not a true mixture since in Gram-stains of the urinary sediment, and in cultures, staphylococci and pyocyaneus were never found together Always one species dominated the other species. Suppression of the staphylococcal infection evealed the pyocyaneus infection, suppression of the pyocyaneus infection revealed the staphylococcal infection and

Table I. Case 1. Changes in bacterial flora during treatment

1954		Pyuria	Gram-stain			Culture				
			Gram neg rods	Gram pos. cocci in pairs	Gram pos. cocci in clusters	Pr. vulgaris	Ps. pyocyanea	S. faecalis	Resist. S. aureus	
March	23	++	−	+++	+	(+)	−	++	+	Pos. blood
April	4									culture, resist.
	5	++	−	+++	+++	+	−	+	++	S. aureus
	7	+	−	−	+++	+	−	−	++	2 g erythro-
	8	+	(+)	−	++	+	−	−	+	mycin daily
	9	+	−	−	++					during 7 days
	12	+	+	−	(+)	+	−	−	−	400 mg nitro-
	13	+	+	−	+	−	+	−	(+)	furantoin
	14	+	++	−	(+)	−	++	−	−	daily during
	15	+	++	−	+	−				8 days
	16	+	(+)	−	−	−	+	−	−	
	17	+	+	−	−	−	+	−	−	2 g strepto-
	20	−	−	−	−	−	−	−	−	mycin i. m. and 4 g sopronal daily during 6 days
	23	−	−	−	−	−	−	−	−	No therapy
	26	−	−	−	−	−	−	−	(+)	
	27	+	−	−	+	−	−	−	+	
	28	++	+	−	++	+	−	−	++	
	29	+	+	−	+					400 mg nitro-
	30	−	−	−	−					furantoin
May	1	+	+	−	−					daily during
	3	+	−	−	−	−	+	−	−	7 days
	4	+	−	−	−	−	+	−	−	
	5	+	−	−	−					No therapy
	6									Pos. blood
	10	++	++++	−	−	−	++	−	−	culture resist. S. aureus
	12	++	+++	−	−	−	++	−	−	2 g erythro-
	13	++	+++	−	−	−	++	−	−	mycin daily
	31	++	++++	−	−	−	++	−	−	during
June	25	++	++++	−	−	−	++	−	−	6 weeks
July	6	++	++++	−	−	−	++	−	−	
	12									1 g oxytetra-
	15	−	−	−	−	−	−	−	−	cyclin and
	17	(+)	−	−	−	−	−	−	(+)	100 mg polymyxin daily during 6 days
	19	+	−	−	−	−	−	−	(+)	No therapy
	21	++	+	−	++++	−	−	−	++	
Sept.	14	++	+++	−	−	−	++	−	−	
Nov	2	++	+++	−	−	−	++	−	−	

Sensitive only to erythromycin and nitrofurantoin. Sensitive only to chloramphenicol.

Table III. Case 2. Results of treatment of urinary tract infection after the second operation

1957		Pyuria	Gram-stain			Culture				
			Gram neg. rods	Gram pos. cocci	Candida	Pr vulgaris	Pr rettgeri	S. faecalis	Candida	
Aug.	6	+	+++	+++	−	++	−	++		
	7	+	++	++	−	++	−	++	−	400 mg nitrofurantoin daily
	8	−	++	++	−					
	10	−	+	+	−	+	−	−	−	
	12	−	++	(+)	−					
	14	−	+	−	−	+	−	−	−	
	19	−	++	−	−	+	−	−	−	
	23	+								400 mg nitrofurantoin, 120 mEq mandelic acid daily
	27	+	+	−	++	+	−	−	++	
	30	+								2 g chloramphenicol daily
Sept.	2	+	+++	−	−	−	++	−	−	
	7	Discharge								
	19	++	+++	−	−	−	++	−	−	60 mEq mandelic acid daily
Nov	14	++	+++	+	−	+	++	+	−	

with the strain cultured before the operation sensitivity tests disclosed the same, somewhat unusual results. During this period, Pr rettgeri was frequent post-operative invader of the urine in the urological ward in vitro the organism was sensitive to neomycin only The patient was discharged and advised to continue treatment with low dosage of mandelic acid to prevent pyelonephritis. Two months after discharge, Pr rettgeri, strain of Pr vulgaris, showing the same sensitivity patterns as before operation, and S. faecalis were cultured from the urine.

In May 1958, the patient was re-hospitalized. H then had blood pressure of 140/90 and the creatinine clearance, which during previous observations had been normal (averaging 110 ml/min) had decreased to 62—70 ml/min. On X-ray examination the shadows of the stones had not become larger There was a pyuria and the same Pr vulgaris and Pr rettgeri were present. During treatment with massive doses of penicillin combined with neomycin, C. albicans reappeared (table IV) Treatment of this latter infection failed. Without treatment C. albicans gradually disappeared while Pr rettgeri again reappeared. C. albicans could again be demonstrated when nitrofurantoin was given in conjunction with mandelic acid. It is possible that mandelic acid may stimulate the growth of candida, since C. albicans appeared in the urine when this drug was administered (cf. tables II and III) The patient was finally discharged with an infection mainly involving Pr rettgeri and pyuria.

Comments

The urinary tract infection of this patient developed during the first operation and, prior to the second operation, was caused by Pr vulgaris, Aerobacter S. faecalis and C. albicans. The failure of antibiotic therapy is attributed to the continual presence of renal calculi. During his second operation an infection

Table II Case 2 Results of treatment of urinary tract infection before the second operation

1957	Pyuria	Gram-stain			Culture				
		Gram neg. rods	Gram pos. cocci	Candida	Pr vulgaris	Aerobact.	S faecalis	Candida	
Jan. 9	++	+++	+++	−	++	++	+	−	
10	++	+++	+++	−	+	+	−	−	4 g chloramphenicol and 400 mg nitrofurantoin daily
11	++	+	++	−	(+)	−	−	−	
14	++	+	++	−	−	−	−	−	
15 16	++	−	+	−	−	−	−	−	
17−23	+	−	+	+++					4×10^4 U penicillin i.m., 90 mEq mandelic acid daily
24	+	−	−	+++	−	−	−	++	
25 26	++	−	−	+++					
28	++	(+)	−	+++	−	+	−	++	150 mEq mandelic acid daily
29 30	++	+++	−	++	−	++	−	++	
31	++	+++	+	+	(+)	++	−	+	
Feb. 1 2,3 4 6	++	+++	+	+	++	++	−	+	
March 12	++	+++	−	−	++	++	−	−	

finally the pyocyaneus infection dominated spontaneously

A similar phenomenon could be observed even more clearly and over a period of 1 ½ years in another patient.

Case 2 21 year-old male. The patient was afflicted with a coxotuberculosis since 1955 and had developed renal calculi during his immobilization. Following nephrolithotomy in Feb 1956 a urinary tract infection developed which did not respond to antibiotic therapy In June 1956, the patient was admitted to our department. On examination, calculi were found in both renal pelves and in the left ureter There was a urinary tract infection blood pressure renal function and calcium metabolism were normal. Despite the presence of calculi the patient was treated for his urinary tract infection

In table II it can be seen that during therapy the flora changed from Pr vulgaris, Aerobacter and S. faecalis to Candida albicans, and that following the discontinuance of treatment the flora changed back to Pr vulgaris and Aerobacter There were no alterations in the sensitivity tests of the isolated bacteria throughout the entire observation period. This lends support to the view that the same micro-organisms were present before and after the long courses of treatment. The Pr vulgaris in this infection was characterized by the fact that the strains were only sensitive to nitrofurantoin and chloramphenicol and not to the sulphonamides, streptomycin and tetracycline

The patient again underwent operation and in three separate operations, carbonate-phosphate stones were removed from the left ureter left kidney and right kidney respectively Despite massive post-operative antibiotic treatment the urinary infection persisted and the patient was readmitted to our department in Aug 1957 Both kidneys were functioning well but radiological examination revealed shadows of a few stones in the region of both kidneys and in the left ureter

The change in flora, as a result of antibacterial treatment, from Pr vulgaris and S. faecalis to Pr rettgeri is depicted in table III The Pr vulgaris was probably identical

with Pr. rettgeri was added. In this mixture of different bacterial organisms one or two species always dominated. The intermittent presence of the same strain of Pr. vulgaris for 1 ½ years was always associated with variations in antibacterial therapy. The presence of C. albicans in urinary tract infections is very uncommon. The fact that C. albicans appeared in this patient's urine on three occasions following large dosages of mandelic acid when few bacteria were present in the urine, leads to the conclusion that very small numbers of C. albicans must have always been present in the urinary tract. The same holds true for Pr. rettgeri, which dominated the infection most of the time after the second operation and whose disappearance during treatment directed against this organism was *more apparent than real.*

Discussion

When broth is inoculated with an equal number of two different bacterial species, the organisms of each species will not remain in numerical equilibrium during culture. The antagonism between the different species is probably chiefly due to competition for nutrients contained in the medium (1) but antibiotic substances known to be produced by bacteria may also be involved. Ps. aeruginosa forms two substances, i. e., procyanine and pyocyanase, both having antibiotic activity. There is some *evidence that* anti-candida substances may also be produced by bacteria (7).

In urinary tract infections, bacteria grow in the urine *in vivo* just as they grow in broth *in vitro*. Those species of bacteria not growing in urine, e. g. tubercle bacilli, do not cause a true urinary tract infection, but an infection of the kidneys, prostate or epididymis, with excretion of bacilli in the urine.

In our experience a change in bacterial flora is seldom encountered when an infection has not been caused by a previous catheterization. Spontaneous infections of the urinary tract are mostly true, single infections. An exception is the rather frequent combination of B. coli or Proteus with S. faecalis, the Gram-negative rods generally dominating. In these instances, when treatment with sulphonamides is instituted, the result will be an infection with S. faecalis. Garrod et al. (2) also mentioned the occurrence of infection with S. faecalis after treatment with sulphonamides. If, in these cases, the Gram-stain of the urinary sediment before treatment is re-examined more thoroughly a few Gram-positive *cocci will be found among* the many Gram-negative rods.

When, however, the urinary tract infection has been caused by catheterization, urological operation or especially following the utilization of an indwelling catheter without taking the necessary precautions, a change in bacterial flora *is often encountered during* treatment. It is easy to understand that in frequent catheterizations or in the use of an indwelling catheter more than one bacterial species will be able to reach the urinary bladder. As described *in the* previous case records, a "mixed" infection can be mistaken for a "single" infection, because the fastest growing bacteria will inhibit the growth of other organisms. (Close examination of the inhibition zones around the paper discs in sensitivity tests sometimes helps in the isolation of additional bacterial *species.*) When the antibacterial agent has succeeded in inhibiting the growth of the predominant organism, other bacteria, pro-

Table II Case 2 Similar changes in bacterial flora on resumption of antibacterial therapy after 8 months without treatment

1958	Pyuria	Gram-stain			Culture				
		Gram neg rods	Gram pos. cocci	Candida	Pr vulgaris	Pr rettgeri	S. faecalis	Candida	
May 29	++	+++	−	−	+	++	−	−	
31									4 × 10⁶ U penicillin i.m., 120 mEq mandelic acid daily
June 2	+	−	−	−	(+)	(+)	−	−	
3 4 5, 6	+	−	−	−	−	−	−	−	
7 9	+	−	−	−	−	−	−	−	4 × 10⁶ U penicillin i.m., 2 × 0.5 g neomycin i.m. daily
10 11 12	+	−	−	++	−	−	−	++	
17–28	+	−	−	+++	−	−	−	++	4 × 10⁶ U penicillin i.m., 4 × 10⁶ U nystatin 120 mEq mandelic acid, also pentamidine i.m. and KJ
29	+	(+)	−	++	−	−	−	+	No treatment
30	+	−	−	++	−	−	−	+	
July 1	+	+	−	+++	−	+	−	++	
2	+	+	−	++	−	+	−	++	
3	+	+	−	++	−	++	−	++	
4	+	++	−	++		++	−	+	
5	+	+	−	++	−	+	−	+	
7	+	(+)	−	−	−	(+)	−	−	
8	+	+	−	−	−	+	−	−	
9	+	+	−	−	−	+	−	−	200 mg nitrofurantoin, 120 mEq mandelic acid daily
10	++	+	−	+++		+	−	++	
11	+	++	−	+	−	+	−	+	
15	+	+	−	+++	−	+	−	++	No treatment
16	++	+++	−	+++	−	++	−	++	
17	++	+++	−	+++	−	++	−	++	
18	+	+++	−	−	−	++	−	−	
21	Discharge								
Aug 21	++	+++	+	−	−	++	+	−	

Acta Medica Scandinavica. Vol. 172, fasc. 3, 1962

From the University School of Medicine, Medical Department A.
(Head: O J Broch, M. D), Bergen, Norway

Chronic Exudative Pericarditis Treated by Pleuro-pericardial Fenestration

By

A. M. Abrahamsen and O J Broch

Exudative pericarditis is as a rule secondary to an acute or subacute illness the aetiology of which may vary greatly An idiopathic group has been proposed in which the cause may be a virus infection or perhaps an autoimmune process. In some cases a chronic pericardial effusion of unknown aetiology has been observed and this has often persisted over several years (3 4 5 7 8, 12, 13 14 15 16)

Pathological changes in the pericardium have not been reported (15) Geneen (7) has described the condition in two brothers. In 1919 Alexander (1) reported a case of chronic pericardial exudate where the pericardial fluid was golden and was found to contain cholesterol crystals. Similar cases have also been observed later (2, 6 7)

With chronic idiopathic pericardial effusion the patients are relatively little affected. Symptoms, like those which come with heart failure, appear but as a rule there is no sign of tamponade. Recurring chest pain and periodic fever have been described (16) Tapping of the pericardial fluid in the usual way is, as a rule, without effect as the fluid collects again quickly Surgical treatment has therefore been tried, and with very good results. Romero (11) in 1819 had already operated on 3 patients with exudative pericarditis two of whom survived.

Several authors have operated on cases of this type (4 5 7 9 10 12, 14 16) Of these Scheuer (12) operated on 14 cases, and Proudfit and Effler (10) on 16 cases.

The types of operation have differed somewhat. The majority have chosen to make a window in the pericardium with drainage to the left pleural cavity This seems to have given satisfactory results. Some (9 14) consider that one is more certain to get good drainage if a slightly larger part of the pericardium is resected, and others have removed the greater part of the pericardium (7 16)

A typical case of idiopathic exudative pericarditis in which good results were obtained with pleuro-pericardial fenestration will now be reported

Submitted for publication March 3, 1962.

vided they are resistant to the drug used will no longer be inhibited in their growth The net result of antibacterial treatment in such cases is frequently the abundant growth of very resistant organisms Pr rettgeri Ps. aeruginosa resistant staphylococci which have reached the urine as hospital infections.

The change in the bacterial flora during antibacterial treatment is described here for infections in a closed cavity i. e. the noncatheterized urinary bladder of male patients. In infections which are not shut off from the outside a similar phenomenon occurs. In examining pus from operation wounds or from burns, or sputa from patients with bronchiectasis, a change in bacterial flora will be found frequently during the course of the antibacterial treatment. In open infections, however the causes of these changes are only partly similar because the alterations can also arise from superinfections with bacteria from the outside. The latter phenomenon was described as replacement phenomenon by Knight and Holzer 1954 (4) in connection with the occurrence of resistant staphylococci in the nose of hospitalized patients during antibiotic therapy

The alternation of the infections following the administration of certain drugs was reproducible. The phenomenon is not rare and is almost exclusively found in patients who have been previously catheterized or who have undergone urological operations.

Mixed infections are assumed to exist in these cases the fast growing species inhibit the growth of slower growing organisms in the urine so that microscopic examination and cultures usually reveal only the presence of the predominant species. When treatment is initiated with a drug effective against the only micro-organisms found, suppression of these organisms will be followed by the growth of one or more species resistant to the administered drug This simulates the appearance of a new infection.

Summary

Repeated alternations of three or four species in the bacterial flora of protracted urinary tract infection during antibacterial therapy are described in two male patients. No operations or catheterizations were performed during the observation periods the urinary tract in these patients was a closed system.

References

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The alternation of the infections following the administration of certain drugs was reproducible. The phenomenon is not rare and is almost exclusively found in patients who have been previously catheterized or who have undergone urological operations.

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During the first post-operative days she was rather weak, probably because of a left sided atelectasis, but she soon recovered. Ten days after the operation the venous pressure was 60 mm of water. The findings at the last check up March 1962 were completely satisfactory. The X-ray findings on 4.2. 1961 before the operation, and on 6.4. 1961 five weeks after the operation are presented in figs. 1 and 2.

Before and after the operation haemodynamic studies were carried out by the dye dilution technique (Evans blue) recorded with an earpiece (table I).

Table I. Haemodynamic studies before and after pericardiectomy

	Before op.	1 month after	6 months after
Cardiac output (l/min)	3.9	8.1	9.9
Stroke vol. (ml)	39	117	153
Appearance time[1]	13.6	9.5	10
Intrathoracic blood vol. (l)	3.7	2.3	3
Total blood vol. (l)	6.4	3.6	3.9

1 sec. from the injection of the dye until it reaches the site of registration.

Discussion

The patient had a chronic exudative pericarditis of unknown aetiology. Previously she had had a pleurisy that was probably tuberculous. Tubercle bacilli were present in a lymph node in the neck when the pericarditis was discovered. The completely clear pericardial exudate which was TB negative in many cultures, and the completely normal histological appearance of the serous membranes are definitely against a specific aetiology. The disease had lasted for several years with constant progression, and the high protein content of the exudate is an indication that the reabsorption mechanism was at fault. Operation was therefore carried out as it was thought that constant drainage to the pleural cavity would help combat the defect in reabsorption. The result was very convincing, and I occurred gradually. As soon as 5 weeks *after the* operation the heart shadow was almost normal. Haemodynamic studies carried out one and 6 months after the operation showed considerable changes compared with those before operation. The findings at the last investigation were considered to be normal. The cardiac output increased by 68 % and this was entirely due to an increase in the stroke volume. The intrathoracic blood volume decreased by 1.4 l after the operation. This was definitely more due to pulmonary congestion than cardiac dilatation, as the pericardial exudate accounted for the greater part of the increased heart volume. The total blood volume decreased by as much as 2.8 l and at the same time the circulation time decreased.

There was also a corresponding clinical improvement, and at the last check up she had no dyspnoea and felt completely well.

Summary

A case of chronic idiopathic exudative pericarditis of at least 6 years duration in a 64-year-old woman is reported. In spite of satisfactory treatment with many pericardial tappings the effusion increased. She showed moderate signs of heart failure, but had no definite symptoms of tamponade. After pleuro-pericardial fenestration, about 6 × 7 cm of the pericardium was excised, the exudate disappeared and she recovered clinically. As soon as 5 weeks after the operation the heart shadow was normal and the findings



Fig 1 X-ray taken before the operation showing considerable pericardial effusion.

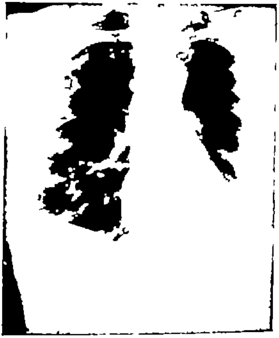


Fig 2. Five weeks post-operatively Some post operative changes are to be seen with a normal heart shadow The heart shadow was unchanged 6 months after the operation.

A farmer s wife born 1897 1940 pleurisy Pirquet + Recovered after 10 weeks. 1950 slight fever of 3 weeks duration accompanied by pain in the left side of the thorax. Enlargement of the heart shadow found on X ray ESR 5 mm At another hospital serous and blood stained fluid was tapped (artifact ?) TB + on culture She was given antituberculous drugs due to the possibility of tuberculosis. Since then in spite of increasing dyspnoea, she managed to carry on with her work to some extent. In 1957 a small enlarged lymph node was found in the left supra clavicular fossa. Pus was found on incision and tubercle bacilli grown (3 colonies) After this she was given further antituberculous treatment.

Her chronic exudative pericarditis continued unchanged with some progression in the size of the heart shadow She was treated in several hospitals and was on seven occasions admitted to Medical Dept. A, Haukeland hospital. She was tapped several times, but the exudate recurred quickly She had moderate dyspnoea on exertion. The liver was palpable under the costal margin. There was no oedema. The area of cardiac dullness was greatly increased. The tones were pure, but distant. On some occasions gallop rhythm was heard. ECG low voltage. BP about 120/80 ESR normal. Blood picture normal. X ray investigation showed a generally enlarged heart with small pulsations. AST 180. Only a few cells were found in the pericardial exudate. Tumour cells — Protein content of exudate 5 6 g/100 ml. Cultures for TB —

In May 1960 cardiac catheterization with angiocardiography was carried out. This showed that the heart chambers were of normal size. The pressure in the right atrium was 5/0 mm. Average pressure 2 5 mm. In Feb. 1961 the venous pressure was 100 ml of water

She was treated with a salt free diet and digitalis, together with pericardial tapping However as her condition had remained unchanged for 5—6 years pleuropericardiotomy was carried out on 1 3. 1961 (by Professor H. G Gade) Both the pleura and the pericardial surfaces appeared completely normal and no adhesions were present. One litre of clear serous fluid was aspirated from the pericardial cavity A 5 × 7 cm piece of the mediastinal pleura and of the parietal pericardium was excised The histological findings in the pleura and pericardium were normal.

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Control of Anticoagulant Therapy

A Comparison of the Thrombotest, the Prothrombin-Proconvertin Method, and the Prothrombin Time

By

C. Fichera

Evidence from studies conducted in many countries has shown that prophylactic therapy with indirect anticoagulant drugs reduces the risk of recurrence in survivors of myocardial infarctions, is effective in preventing infarction in angina pectoris, and also prevents thromboembolic complications in rheumatic heart disease (1, 2, 3).

During the past ten years, therefore, treatment with anticoagulants has steadily increased, and many patients are now dependent on laboratory control of the coagulability of blood for protection against thrombosis formation and abnormal bleeding during treatment. As a consequence, increasing importance has been given to the methods of control.

In this paper three laboratory methods for the control of anticoagulant therapy — the thrombotest, prothrombin-proconvertin time, and prothrombin time — are compared and their practical implications discussed.

Submitted for publication March 4, 1962.

Material

Blood from healthy normal subjects and from patients under anticoagulant treatment with phenylindanedione was studied.

Thrombotest reagent was kindly supplied by Nyegaard and Company, Oslo, in vacuum-sealed ampoules, stable at room temperature for one year. This "all-in-one" reagent has been designed for sensitivity to all four coagulation factors affected by anticoagulant treatment: prothrombin, factor VII (proconvertin), factor IX, and factor X. This object is thought to have been achieved with a preparation that consists of: a) highly active cephalin preparation corresponding roughly to platelet factor 3; b) a tissue thromboplastin extract of low activity; c) a stabilized bovine plasma completely free of the four factors to be measured; and d) $CaCl_2$ in optimal concentration.

Thrombokinase was kindly supplied by Geigy AG, Basel. It is thromboplastin from calf lungs with an optimal quantity of $CaCl_2$ (0.18 %) added. This reagent was used for all estimations of prothrombin time.

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were unchanged when she was last observed one year after the operation. The records taken one and 6 months after the operation showed marked haemodynamic changes. The cardiac output and the stroke volume increased by two thirds. The intrathoracic and total blood volume were reduced and the circulation time decreased. Observations on the patient about 12 months after the operation showed that she is still well.

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In 40 cases capillary blood obtained from finger tip puncture with a sharp blade was studied.

The standard reference plasma for the correlation curves was obtained by pooling plasma prepared from five donors with normal haematocrit (45—47 %) silicone technique was used. The plasma was stored in aliquots in stoppered plastic or siliconized tubes at — 25 C. The same plasma was used for all the standard curves.

Prothrombin-proconvertin time (Owren and Aas 1951 (4)).

A mixture consisting of 0.2 ml saline-extracted human brain thromboplastin, 0.2 ml ox plasma reagent, and 0.2 ml test plasma diluted 1 10 was incubated for 5 min. in a water bath at 37° C. The clotting time was recorded after the addition of 0.2 ml calcium chloride 0.030 M. All determinations were made in duplicate.

Prothrombin time (Quick 1935 (6))

The thrombokinase tablet was dissolved in distilled water and incubated 20—30 min. at 37° C. 0.1 ml of citrated plasma was kept 2 min. at 37° C 0.2 ml of thrombokinase suspension was then added, and the clotting time recorded. All determinations were made in duplicate

Thrombotest (Owren 1959 (7))

The lyophilized reagent was dissolved in calcium chloride solution of 3.2 mM that compensated for the citrate in the blood sample. 0.25 ml of the reagent was measured into small test tube and left in the water bath at 37° C for minimum of 3 and maximum of 30 min. to reach the correct temperature. Citrated venous blood, 0.05 ml, was aspirated into pipette and mixed with the reagent and the coagulation time determined. When capillary blood was used the reagent was dissolved in distilled water and 0.05 ml of blood was added to 0.25 ml of the reagent.

I all the tests, including the prothrombin time, the coagulation time was converted into coagulation activity expressed as percentage of the normal, with the aid of correlation curves derived from the normal standard plasma. Serial dilutions of normal standard plasma in adsorbed plasma were tested with thrombokinase and the resulting correlation curve was identical with the curve supplied by Geigy

Table I

	Prothrombin time with	
	Human brain	Thrombokinase
Normal human plasma	13 sec.	15 sec.
Proconvertin-deficient plasma	83	22
Factor X-deficient plasma	50	34

Results

The prothrombin time and the prothrombin-proconvertin time (PP) were measured in 259 samples prothrombin time and thrombotest (TT) in 134 samples and prothrombin time, PP and TT in 71 samples.

The thrombotest was performed in the first 40 cases on citrated blood, citrated plasma, and capillary blood The same results were obtained and citrated blood was therefore used for the following investigations.

There was fair correlation between TT and PP for values in the therapeutic range (fig 1)

Comparison of prothrombin time with PP and TT showed a consistently higher percentage value of prothrombin activity than of TT and PP (figs. 2 and 3) for patients on anticoagulant therapy

The sensitivity of the thrombokinase reagent to proconvertin and factor X was determined by testing normal, proconvertin-deficient, and factor X-deficient plasmas. The results indicate that the reagent is relatively insensitive to proconvertin and to factor X (table I)

Discussion

The thrombotest was simple and reliable, giving clotting times that were reproducible with a sharp unmistakable

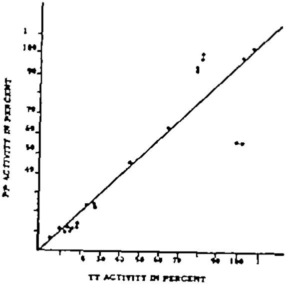


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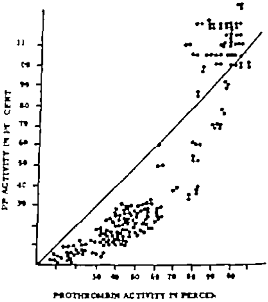


Fig 3

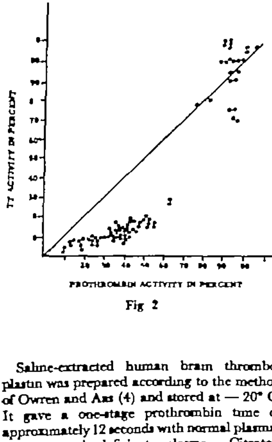


Fig 2

Saline-extracted human brain thromboplastin was prepared according to the method of Owren and Aas (4) and stored at — 20° C. It gave a one-stage prothrombin time of approximately 12 seconds with normal plasma.

Proconvertin-deficient plasma Citrated plasma (9 parts of blood and 1 part of 3 13 % sodium citrate dihydrate) from a patient with congenital lack of proconvertin was stored at — 20° C. It contained approximately normal amounts of prothrombin and proaccelerin.

Bovine-absorbed plasma Citrated ox plasma was filtered once through 20 % asbestos filter pads and once through 50 [illegible] asbestos filter pads (4) The plasma was stored at — 20° C.

Dilution fluid A dilution fluid with pH 7 35 and ionic strength of 0 154 was prepared according to Hjort et al. (5) by mixing 200 ml of veronal buffer 200 ml of 25 66 mM sodium citrate, and 600 ml of 0.9 mg/% sodium chloride solution.

Calcium chloride A 30 mM solution was made by dilution of a 1 M aqueous stock solution with distilled water

Methods

Collection of blood Citrated blood (1 vol. of 3 13 % sodium citrate dihydrate to 9 vol. of blood) was used. The blood specimen was taken with a syringe in which the amount of citrate solution had been accurately measured, and the mixture was then transferred to a small test tube.

Because thrombotest is sensitive to contact activation of the test sample plastic or siliconized test tubes were used to avoid false high values.

To obtain plasma, the blood was centrifuged at 2,500 rpm for 20 min. in a refrigerated (+ 4 [illegible]) international centrifuge.

In 40 cases capillary blood obtained from finger tip puncture with a sharp blade was studied.

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Discussion

The thrombotest was simple and reliable, giving clotting times that were reproducible with a sharp unmistakable

endpoint. Theoretically it appears to be adequate since it reflects the total defect induced in the clotting system of the patient under anticoagulant treatment. It showed *some advantages when compared* with prothrombin time and PP

The one stage prothrombin time gave very short clotting times and more technical experience is therefore required to perform it. Because of the short interval between 100 % and 50 % of the prothrombin complex there is some risk of overdosage in the first days of treatment (8) Moreover there are important differences caused by varying sensitivity to the reduction of factors VII and X. Different thromboplastins prepared in different laboratories, therefore, may give quite different results with the same plasma. Furthermore the prothrombin time is influenced by the concentration of factor V (proaccelerin) in the plasma tested.

The PP method is somewhat more sensitive than the prothrombin time method Because proaccelerin and fibrinogen are present in the substrate plasma, the test gives only a measure of proconvertin, factor X, and prothrombin It has the disadvantages that *it is not sensitive* to factor IX and is not simple enough.

The accuracy of the PP method in which some sources of error are eliminated by dilution of the test plasma, could not be achieved when the TT method was used since the determinations were made with undiluted samples. The difference, however is probably of little importance in clinical use.

The findings of Owren (1959) that TT and PP gave similar results were confirmed. This was particularly evident in the cases of patients on anticoagulant therapy since the TT values were obtained from the part of the correlation curve that has the most convenient gradient for accurate reading In the normal range, however a difference of a few seconds in the registered thrombotest times means *a considerable difference in the coagulation* activity when expressed as a percentage of normal standard plasma because of the shape of the correlation curve.

The higher values for the prothrombin time, as compared to TT and PP were presumably due to the low sensitivity of the thrombokinase reagent to proconvertin and factor X. During long term anticoagulant therapy factor X is usually lower than prothrombin (9) the Geigy test is primarily a test for prothrombin. The TT and PP both of which are sensitive to factors X and VII will show the lowest results. The one stage *prothrombin time showed great variations* in percentage activity when multiple tests were performed on the same sample because of the flat reference curve. The difference between 100 % and 5 % activity is only about 45 sec. while the TT and PP reference curves are steeper with a difference of about 200 sec. between 100 % and 5 %

In patients controlled with the prothrombin time method the so-called therapeutic range has been given as 30 % to 18 This corresponds to thrombotest values which on an average, are somewhat lower than the recommended optimal level of 15 % with a range of 10 % to 25 % This again results from the fact that the thromboplastin used was insensitive to factors VII and X. When such thromboplastins are used bleeding caused by factor X deficiency could occur without being preceded by a prolongation of the prothrombin time above the therapeutic level (10)

Summary

The thrombotest has certain theoretical advantages in that it gives a combined measure of all the factors influenced by anticoagulants. The practical advantages are obvious. It can be performed on citrated blood or citrated plasma and on capillary blood. The last is particularly easy to perform, and the equipment needed is very simple. Because of the steep reference curve and the sharp endpoint, the reading of the activity as a percentage of normal is accurate for patients in the therapeutic range of anticoagulant therapy and not too much technical experience is required for the performance of routine tests. The reagent is stable, and the results are reproducible and comparable with those of other laboratories.

A comparison of the three tests studied showed that the prothrombin time test using thrombokinase Geigy gave higher values than the TT and PP. The reason presumably is the low sensitivity to factor X and factor VII of the thrombokinase.

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Ruptured Aneurysm of Sinus of Valsalva

Report of two Cases

By

Edvar Wolff Sorensen and Leif Kollaker

The cusps of the aortic valve and the adjacent sinuses of Valsalva can be named according to their relationship to the coronary arteries. Rupture of a sinus of Valsalva is said to occur on the basis of a preceding aneurysm (3). Aneurysms of the sinuses of Valsalva are rare. Snyder and Hunter (18) found only 10 cases in 1,200 autopsies, and similar figures have been given by Schuster (16). Most of these aneurysms are congenital (22) and are the result of a break in the continuity of the aortic media and the annulus fibrosus of the aortic valve (5). In other cases the aneurysm formation may be caused by syphilis, arteriosclerosis or bacterial endocarditis. The rupture usually occurs into the right atrium and the right ventricle (8). In a review of the world literature in 1957 Sawyers et al. (15) found 49 autopsied cases of rupture of aneurysms of sinus of Valsalva, 34 (70 %) of which had ruptured from the right coronary sinus. Of these 34 cases 19 (70 %) had ruptured into the right ventricle. In 13 cases the aneurysm was found to be in the "non coronary sinus" with rupture to the right atrium. Rupture of aneurysms of the sinus of Valsalva is 4 times commoner in men than in women, and the patients' age is on an average about 32 years (15).

In the absence of complications an aneurysm of sinus of Valsalva gives rise to no characteristic signs or symptoms (20), but the diagnosis can, in some cases, be made on angiocardiography (20). Rupture of an aneurysm of sinus of Valsalva is often provoked by physical exertion (3, 9, 12), but may occur at any time. Such a rupture gives an acute syndrome characterized by severe precordial pain, severe dyspnoea and collapse (3, 12, 14, 15). On auscultation of the heart a systolic and a diastolic murmur can be heard with the point of maximum intensity over the left sternal border in the 3—4 intercostal space. The murmurs recall those heard with a patent ductus arteriosus of Botalli or a ventricular

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Fig 1 Case 2 X-ray of heart and lungs.

septal defect. Electrocardiography is of little use diagnostically (12 14) The diagnosis can be verified with the help of aortography (2 4 6 13) angiocardiography (14 20) and cardiac catheteriza tion.

The prognosis in rupture of a sinus of Valsalva depends on the size and locali zation of the rupture. The survival time after the occurrence of the rupture may vary from seconds (9) to weeks, and one case has been reported with a survival time as long as 17 years (8) It is stated that 50 % of cases die within the first 2 months, and very few live longer than 3 years.

Rupture of aneurysms of the sinus Valsalva is seldom diagnosed in life. Successful operations on the open heart during hibernation anaesthesia with closure of the rupture have been carried out (1 7 10 15) Spencer et al (19) describe the 10 patients who had been operated upon after these principles prior to April 1960 and in all of whom the result had been successful.

Case reports

Case 1 The patient was a 75-year-old man who had previously been in good health and a strong workman Previous heart examinations had shown nothing of note until he was 65 years of age. He had then become acutely ill while hurrying to a shelter during an air-raid. He collapsed, and on examination was found a systolic and a diastolic murmur of consider able strength over the sternum. Normal blood pressure. Later there were found to be slight-doubtful- signs of congestive failure, but the patient was not troubled by dyspnoea, cyanosis or oedema. He was admitted to hospital because of weightloss and melaena.

Physical condition. The patient had no oedema, no cyanosis or venous congestion. He had auricular fibrillation without pulse deficit. Blood pressure 150/80 mm Hg Serological examinations for syphilis negative. The apex beat was of a heaving type and in the 5th intercostal space in the anterior axillary line. Over the apex a systolic murmur of strenght 3—4 was heard, and this increased to strength 4—5 on the left sternal margin in the 3rd intercostal space. In addition a long-drawn-out diastolic murmur was heard over the left sternal margin, strongest in the 3rd intercostal space. The 2nd sound was accentuated in the pulmonary area. The electrocardiogram showed auricular fibrillation and left preponderance. The arterial oxygen saturation was 94 6 % X-ray investigation of the heart showed moderate, almost concentric enlargement. The aorta was wide, looped with calcified cusps and strong pulsation in the ascending aorta and the aortic arch. There were no signs of congestion or oedema in the lungs.

Laparotomy was carried out, and the patient was found to have an inoperable gastric carcinoma. He died one month later At autopsy performed at the Gade Institute, Department of Pathology (E. Waaler M.D.), Bergen, the follow g findings appeared (only findings of interest will be mentioned)

Heart Weight 480 g Some hypertrophy and dilatation, particularly of the left ventricle. The aortic cusps were stiff and calcified. The right sinus of Valsalva was considerably dilated and had ruptured. There was a communication almost as thick as a pencil, with the right ventricle just below the origin of the

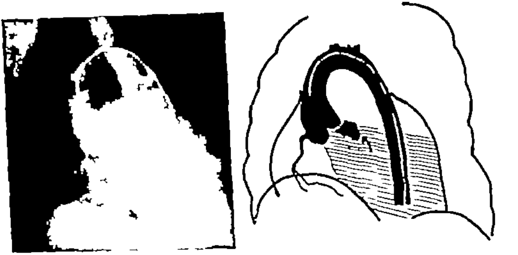


Fig. 2. Case 2. Thoracic aortography

pulmonary artery. There was nothing of particular note about the mitral cusps.

Aorta. Marked calcification and ulceration. No break in the continuity of the elastic fibres was found on microscopic examination.

Conclusion. Aneurysm of the right sinus of Valsalva with rupture into the right ventricle. No evidence of syphilitic or rheumatic disease was found. The aneurysm was probably of congenital origin.

Case 2. The patient was 37-year-old man who had previously been in good health. About week before admission to hospital he got sudden attack of compressing and constricting pain localized to the epigastrium and thorax. This was attributed to epidemic myalgia. He stayed in bed for 3—4 days. On the day of admission, Feb 2nd, 1961 the patient was found lying disorientated, at times screaming, at times delirious. He appeared to be in great pain.

Marked cyanosis of the upper part of the thorax, neck, head and fingers and congestion of the neck veins. Blood pressure 75/50. Pulse 136/min, regular. Pulsation was found to be symmetrical in both the femoral and carotid arteries. The apex beat could not be palpated with certainty. The heart sounds were pure and the action regular. There were no signs of peripheral oedema or pulmonary congestion. Ophthalmoscopy findings normal. Hb 93 %. ESR 5 mm. WBC 6,000. Urine physiological. Blood urea 50 mg %. Venous pressure 170 mm. Spinal fluid: physiological. ECG normal. The values for the serum-glutamic-oxalacetic-transaminase for the first 4 days following admission were 1,600, 1,600, 150 and 90 units.

During the admission a slight right-sided hemiparesis developed. The ECG showed nothing that was definitely pathological. On auscultation of the heart few days after admission there was found to be systolic murmur of strength 3—4 and a loud diastolic murmur of maximum intensity in bout the 3—4 intercostal space on the left of the sternum, decreasing towards the base of the heart and the apex. X-ray investigation of the heart few days after admission showed diffuse enlargement of the heart shadow with weak pulsations. Fluid in the left pleura. Blood was tapped from the pericardium. Thoracic aortography was carried out with catheterization from the right femoral artery by Seldinger method (17). The end of the catheter was located 4 cm from the aortic ostium. The angiograms (in the left oblique position) showed a grape-sized collection of contrast medium on the left side of the heart (left ventricle). The X-ray of the thoracic aorta gave no indication of the presence of dissecting aortic aneurysm. X-ray diagnosis probable aneurysm of the sinus of Valsalva with rupture int the left ventricle (fig. 2).

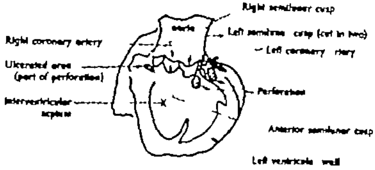


Fig. 3 Case 2. Schematic diagram of the pathologico-anatomical findings.

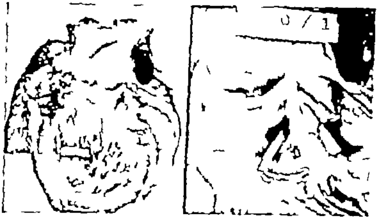


Fig 4 Case 2. Pictures of the pathologico-anatomical preparations. The penings of the perforations are marked with sticks.

The condition was diagnosed as that of an aneurysm of the sinus of Valsalva wh ch had ruptured into the left side of the heart and pericardial cavity The patient was transferred to Surgical Dept A. (Leif Efskind M D) Rikshospital, Oslo, 4 weeks after admission to hospital.

The patient had several attacks in which he collapsed and became withdrawn with a very weak, fast pulse and unrecordable blood pressure The pericardium was punctured and blood aspirated. His condition deteriorated rapidly he became unconscious and cyanotic Thoracotomy was attempted under intubation anaesthesia. The pericardium was opened, and blood removed — partly clotted and partly normal At the same time arterial blood also flowed out. Cardiac tamponade was tried. The heart action stopped, but started again after massage but the bleeding continued. The condition became irreversible and the patient died on 22.3 61

At operation it was observed that the bleeding had clearly come from the intra pericardial part of the heart, up by the origin of the aorta. A ruptured sac could be seen, and a pea-sized opening into a blood filled cavity

At autopsy performed at the Rikshospital pathological laboratory (Olav Torgersen, M. D) Oslo, was found (only findings of interest will be recorded)

Pericardium Much infused with blood and covered with adhesions which look like clot or partly organized thrombi. No definite opening into the pericardium, as described at operation, was found.

Heart Weight 510 g On opening the heart n the usual way the left semilunar cusp was divided into two (figs 3 and 4) A perforation was found in the posterior wall of the left ventricle just under the aortic cusps, and at the point where the right semilunar cusp touches the left semilunar cusp. The perforation had an irregular ragged edge and measured 15 × 12 mm. In addition a second perforation was found which was somewhat smaller and which lay n the centre of the cut surface at the lower edge of the left semilunar cusp. This appeared to be a small perforation or communication from the aortic side of the cusp down into the heart wall. Both the above-mentioned perforations led into a *hole* the size of a hen s egg and this was found to be full of blood There was no perforation

from this hole to the atria or to the right ventricle. No ventricular septal defect. The left semilunar cusp was greatly thickened and fibrous, while the right semilunar cusp and the anterior semilunar cusp appeared normal macroscopically On microscopic examination the left aortic cusp was found to be thickened, fibrous and somewhat vascularized. In the areas around the two defects in the heart there were found to be areas containing fibrin and leucocytes. These areas were in part necrotic. Further out in the myocardium similar larger and smaller necrotic areas were found. Foreign-body type giant cells were found in some parts of these necroses, no epithelioid cells, no Aschoff cells. No part icular perlarteritis round the small vessels. In series of sections from other parts of the myocardium no sign of rheumatic or other inflammation was found. No vegetations were found on the aortic or any other cusps and no bacteria were found on Gram-staining

On investigation of the brain there were found to be multiple embolic infarcts.

Autopsy diagnosis. Thickened left aortic cusp with chronic inflammation. Two defects (perforation of the myocardium) in the posterior wall of the heart up towards the aortic ostium. An egg-sized subpericardial bleeding (pseudoaneurysm?) behind the upper part of the heart with large communication with the left ventricle and small communication from the base of the left aortic cusp. Perforation from the above aneurysm into the pericardial cavity Multiple embolic cerebral infarcts.

Discussion

A rupture of sinus of Valsalva was found in both the cases reported, but from the point of view of the history as well as clinically and pathologico-anatomically there were striking differences.

The history and clinical picture in patient no. 2 are characteristic. The course of the illness was stormy with severe pain in the chest, collapse, characteristic findings over the heart and characteristic X-ray findings with perforation to the left ventricle.

In patient no. 1 the diagnosis of rupture of the sinus of Valsalva was made at autopsy but it is considered to be most likely that the rupture had occurred about 10 years earlier in connection with exertion. It is surprising that the patient had very slight symptoms of heart failure, and died of another disease.

The pathologico-anatomical findings were, as mentioned, very different in the two cases. The findings in patient no. 2 were extremely unusual and the case can best be described as a rarity The pathologico-anatomical findings in patient no. 1 were of the type usually found with a rupture of an aneurysm of the right sinus of Valsalva into the right ventricle.

The aetiology of the ruptures described is uncertain. In patient no. 1 there may possibly have been a congenital aneurysm. No inflammatory reaction was found. In patient no. 2 there was a chronic inflammation of the aortic valve. This was interpreted as a probable bacterial endocarditis.

Summary

Two male cases of rupture of the sinus of Valsalva are described One of them lived for 10 years after the rupture took place without — or with slight — symptoms of cardiac insufficiency This man died of gastric carcinoma and the diagnosis of a ruptured sinus of Valsalva was made at autopsy

In the second case the symptoms were characteristically severe, and the patient died within 5 weeks of his first symptoms. The diagnosis was made by means of clinical examination and thoracic aortography The patient died before operative closure could be carried out due to pericardial haemorrhage and cardiac arrest. At autopsy there was found to be chronic

inflammation of the left aortic cusp two defects in the myocardium a subpericardial pseudoaneurysm with a large communication with the left ventricle and a smaller one from the base of the left sinus af Valsalva.

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The Cytology of Colonic Secretion in Proctosigmoidal Disease

By

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In a preliminary communication (2) we described a method for qualitative and semiquantitative cytological examination of the mucosal secretion from the rectum and the part of the colon which can be reached using a sigmoidoscope.

The present paper is an analysis of a series of cytological preparations (appr. 700) obtained by this method from normal subjects and patients with proctosigmoidal disease.

Method and material

The method employed was as previously described. Its principle is that a replica is taken from a circumscribed area of the mucosa. As a rule two samples were taken from each subject.

One hundred and ninety-seven persons in all were examined. Table I shows the distribution of the subjects into clinical groups, the classification having been made disregarding the results of the cytological examinations.

Most of the patients with haemorrhagic proctitis and ulcerative colitis were examined several times, both in different phases of the natural course and during treatment. Several patients from the group chronic constipation — irritable colon were examined before and after conventional treatment including diet and tannic acid enemas.

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Results

Usually colonic secretion from *normal subjects* contained no inflammatory cells at all. Only strands of mucus, a few desquamated epithelial cells and varying numbers of bacteria were found (fig. 1). Occasionally a few neutrophilic granulocytes were seen singly or in isolated small clusters. In these few cases the larger area of the preparations had a completely normal appearance and the inflammatory cells were only found in one of two corresponding preparations.

In a few preparations from normal subjects and from patients of the different categories single clusters of lymphocytes have been found, presumably on account of contact with a lymph follicle. Varying numbers of erythrocytes have occasionally been observed indicating artificial lesion during sigmoidoscopy.

Tabl. I Distribution of patients into clinical groups

Normal subjects	46
Chronic constipation — irritable colon	48
Haemorrhagic proctitis	11
Ulcerative colitis	24
Diverticulitis of the colon	6
Acute infectious enteritis	5
Patients with uncharacteristic intestinal symptoms	40
Uraemia	6
Rectal cancer	4
Various other diseases	7
Total	197

Nine patients were examined before and after treatment of helminthiasis with filix mas and vigorous purgation with laxatives and enemas. In all cases the mucosa was macroscopically and cytologically normal before treatment Immediately after the treatment varying numbers of neutrophilic granulocytes, several lymphocytes and a great many desquamated epithelial cells were seen in the preparations from all but one of these patients. A few eosinophilic granulocytes were found in only one patient. In several cases moderate mucosal redness was observed at sigmoidoscopy, but the inflammatory changes described were also seen in combination with a macroscopically normal mucosa.

In 38 of the 48 patients in the group *chronic constipation — irritable colon* the preparations contained no inflammatory cells at all. In nine, as in some of the normal subjects, a few neutrophilic granulocytes and lymphocytes were found singly or in small clusters. One patient showed numerous neutrophilic granulocytes in a corner of one replica while the other was quite normal. In most of these patients the mucosa was macroscopically normal but a few showed slight pathological changes (redness and granulation) However no relationship between these macroscopical findings and the slight cytological changes described above was present.

Twelve of the patients with chronic constipation — irritable colon were examined before and after conventional treatment with diet and daily tannic acid enemas. In eight the preparations after treatment showed no inflammatory cells, in three a few granulocytes and lymphocytes were found.

The conclusion is that the cytological findings in patients with chronic constipation — irritable colon are identical with those obtained in normal subjects.

The 24 patients with *ulcerative colitis* and the eleven with *haemorrhagic proctitis* showed identical cytological pictures only in two patients — both with haemorrhagic proctitis in quiescent phase — were the changes so mild that any variation from normal was difficult to distinguish. In all other cases pronounced signs of inflammation were present (figs. 2 and 3) These were numerous neutrophilic granulocytes spread diffusely throughout the preparations, varying numbers of lymphocytes and erythrocytes and *as a dominant feature a considerable admixture of eosinophilic granulocytes* In most untreated cases this local eosinophilia was very marked as the number of eosinophilic cells far exceeded that seen in non-allergic inflammatory reaction, for example in the skin (6) Part of a cluster of eosinophilic granulocytes is shown in fig 4 Only in six patients was local eosinophilia mild or absent.

Eosinophilia in the peripheral blood (here defined as more than 400 eosinophilic cells per μl) was found in only 6 patients from these groups, all with ulcerative colitis.

Remission induced by local treatment with hydrocortisone was accompanied

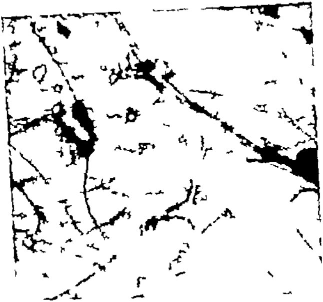


Fig. 1. Mucosal secretion from normal subject. Mucus strands and scattered bacteria; no inflammatory cells. Appr. × 200



Fig. 2. Preparation from patient with proctocolitis. Large numbers of inflammatory cells. Appr. × 200.

by a decrease in the total number of inflammatory cells and the disappearance of erythrocytes. The improvement in the cytological picture usually started proximally and gradually extended distally. The percentage of eosinophilic cells was, however, found to remain high in most cases, even during prolonged treatment. In several cases the cytological picture at the end of treatment was almost normal. Often, however, distinct microscopic signs of inflammation were present in spite of negligible macroscopic findings and approximate freedom of symptoms.

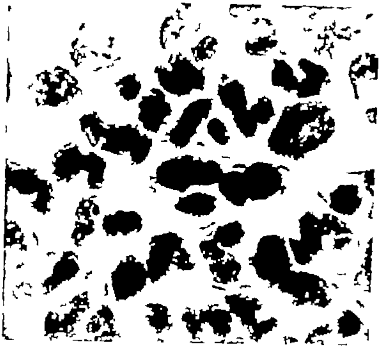


Fig 3 Inflammatory granulocytes and solitary lymphocytic cell from patient with proctocolitis. Appr ×2 000

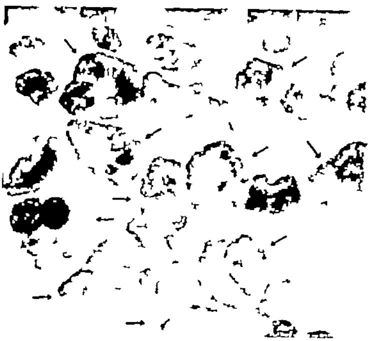


Fig 4 Cluster of eosinophilic granulocytes from patient with proctocolitis. Appr × 2,000.

In such cases the inflammatory cells were located corresponding to the openings of the crypts.

Three of the patients with *diverticulitis of the colon* showed a perfectly normal cytological picture, while preparations from the other three contained several neutrophilic granulocytes spread all over the preparations or concentrated in small clusters. However there was a marked difference between these mild signs of inflammation and the severe inflammatory reaction, which characterized the mucosal secretions of the untreated cases

of ulcerative colitis and haemorrhagic proctitis. In the preparations from four of the five patients with *acute infectious enteritis* inflammation was observed of about the same degree as that seen in the diverticulitis patients whereas no inflammatory cells were seen in one. In neither of these two groups was local eosinophilia found.

Forty patients with *uncharacteristic intestinal symptoms* were examined. Most of these patients suffered from diarrhoea or rectal haemorrhage. Several were referred from other departments for cytological examination because of suspected proctocolitis. Thirty patients in this group had completely normal mucosal secretion. Nine patients showed moderate inflammatory changes with mostly neutrophilic granulocytes, while the number of eosinophilic cells was small. The samples from two patients contained many neutrophilic as well as eosinophilic granulocytes.

The groups *uraemia, rectal cancer* and *various other diseases* (e. g. systemic lupus erythematosus, malabsorption syndrome and erythema multiforme with intestinal localization) are still too small for close analysis. However our experience is, that even severe uraemia does not give rise to inflammation of the colonic mucosa, and that the method has no diagnostic value in rectal cancer because of the often accompanying severe inflammation.

Discussion

Compared with biopsy techniques the method has the following advantages. It is simple and painless and does not represent any risk to the patient.

The method has three main fields of application 1) the differential diagnosis of non-neoplastic diseases in the rectum and distal part of sigmoid colon 2) the assessment of the course of proctocolitis — with and without treatment — and 3) aetiological study of proctocolitis (ulcerative colitis and haemorrhagic proctitis)

The importance of the method in differential diagnosis rests mainly on the possibility of a clear distinction between slight degrees of proctocolitis and non inflammatory states with similar symptomatology. Thus, in a group of patients with distal bowel symptoms the replica method has been able to verify the clinical diagnosis of proctocolitis in all cases.

In the group of patients with chronic constipation — irritable colon the cytological picture was identical with that found in normal subjects. Consequently diagnostic terms indicating an inflammatory state in the colonic mucosa are not justified in connection with this syndrome.

In accordance with general experience purely clinical findings have *only* enabled the tentative diagnosis diverticulitis of the colon. In three of the six patients this tentative diagnosis was supported by the finding of inflammatory cells in the mucosal secretion. The concentration of the inflammatory cells in some mucus strands separated by completely free, normal intervals may be a characteristic feature of diverticulitis.

Forty patients in this study had intestinal disease which could not be definitely classified or clinical criteria. In this group — from which neoplastic and bacterial disease could be excluded — the cytological examination did not permit an exact classification to be made except in two cases, in which the cytological picture was strongly suggestive of proctocolitis. However the method was of clinical value in excluding inflammatory aetiology in most of these cases.

The examination of patients with

helminthiasis before and after treatment with filix mas and purgation demonstrated how promptly quite severe inflammatory changes can follow irritation of a normal colonic mucosa The results emphasize the necessity of not using purgation before cytological examination

The relationship between the macroscopic evaluation of the mucosa through the sigmoidoscope and the result of the cytological examination is of importance for the differential diagnostic value of the method A comparison has been made in all cases examined and has shown — in patients with *slight* symptoms irrespective of diagnosis — that all combinations of macroscopically and microscopically normal and pathological findings are possible. In patients with *severe* symptoms of inflammatory disease however the cytological picture has always been in accordance with the macroscopic findings, but the cytological method in these cases offered a greater possibility of graduating their severity

This possibility of graduation has been used to follow the changes in proctocolitis activity in most of the patients from this group A close relationship between the cytological picture and the clinical condition was found while the macroscopical evaluation only gave a rough estimate of the activity of the process

The demonstration of a large number of eosinophilic granulocytes in the mucosal secretions of most of our patients with proctocolitis has been surprising though local eosinophilia has previously been described in some cases of ulcerative colitis. Thus, by examining 119 biopsies obtained from patients with ulcerative colitis Lumb and Protheroe (5) found that excessive infiltration by eosinophils is inconstant at all stages it was seen in a third of the cases in this series Goldgraber et al (4) found tissue eosinophilia in 40 % of specimens removed at operation and/or autopsy from patients with ulcerative colitis. In a patient with ulcerative colitis and assumed milk allergy Truelove (9) found a considerable number of eosinophilic granulocytes in a biopsy from the colonic mucosa also of interest in this connection is Rissager's (7) demonstration that a rise in the number of eosinophilic cells in peripheral blood preceded and accompanied exacerbations in the course of ulcerative colitis in most cases. On the other hand Dick and Grayson (3) who examined biopsies from 62 patients with proctocolitis — in which 48 showed pathological changes — found only neutrophilic granulocytes, plasma cells, monocytes and lymphocytes, while eosinophilic granulocytes were not mentioned

Our finding of marked local eosinophilia in proctocolitis has naturally led to aetiological considerations. Firstly the identical picture in most of the patients in this group supports the theory that haemorrhagic proctitis and ulcerative colitis represent different degrees of the same disease. Secondly it is tempting to suppose that the local eosinophilia indicates an allergic aetiology Such a working hypothesis is in accordance with the opinions of Andresen (1) and Rowe (8) It appears from examinations of one of our patients, in whom transverse colostomy had been performed for ulcerative colitis, that the pathogenetic mechanism is not a simple reaction to an allergen passing through the lumen of the gastrointestinal tract. Pronounced local eosinophilia was demonstrated in the secretion from the rectal mucosa, which was no longer in contact with the contents of the digestive tract.

In a larger series of proctocolitis patients it may be possible to subdivide the entity according to absence or presence of local eosinophilia.

Summary

An analysis of approximately 700 cytological preparations obtained by a replica method has shown 1) that the colonic mucosal secretion from normal subjects contained few or no inflammatory cells 2) that strong mechanical and toxic irritation of normal colonic mucosa as a rule provoked a definite inflammatory reaction 3) that the cytological findings in patients with irritable colon were identical with the picture seen in normal subjects 4) that treatment of patients with irritable colon with diet and daily tannic acid enemas did not influence the mucosal secretion 5) that the cytological picture in patients with ulcerative colitis and haemorrhagic proctitis was identical large number of inflammatory cells, which was usually reduced during local treatment with hydrocortisone 6) that a great number of eosinophilic granulocytes was found in most of these patients 7) that the secretion from patients with diverticulitis of the colon and acute infectious enteritis usually showed signs of inflammation, but considerably less pronounced than in secretion from proctocolitis patients 8) that the preparations from most of the patients with uncharacteristic intestinal symptoms showed no microscopical inflammatory signs.

The method is simple, free of risk and can therefore be used for frequently repeated examinations.

The cytological principle of the method makes it possible to distinguish between inflammatory states and functional proctosigmoidal disorders.

The method is better suited for following inflammatory activity during treatment of proctocolitis or during spontaneous course of this disease that is macroscopical examination at sigmoidoscopy Cytological preparations often show distinct inflammation in spite of negligible macroscopical findings.

The demonstration in the present investigation of local eosinophilia in most of the patients with proctocolitis may be valuable in aetiological studies of this disease.

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Effect of Sodium Malonate on Gastric Acidity

Preliminary Report

By

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During acid secretion the succinic dehydrogenase activity increases significantly in the parietal cells (3). Since malonic acid and malonate block specifically succinic dehydrogenase, it appeared to be worth while to study the effect of perorally administered sodium malonate on acid gastric secretion. The following is a preliminary report of the results obtained.

Results

A sample of the gastric contents was taken from fasting test subjects, followed by a subcutaneous injection of betazole hydrochloride (Histalog® Lilly) at the same time sodium malonate was given perorally in 0.5 g tablets (Leiras, Turku). The dose was at first 1 g of malonate, but as it appeared to have no effect on the first three test subjects the dose was raised to 2 g and in some cases to 3 g. After betazole hydrochloride, samples were taken at 20, 40 and 60 min., and the pH of the samples was determined (table I).

None of the 13 test subjects had definitely higher pH of the gastric contents after betazole hydrochloride stimulation when the patients had simultaneously ingested 2 g of sodium malonate. In four of these cases the pH was above 6 throughout. Of the remaining test subjects one (No. 11) showed only a mild effect on the pH after 2 g of malonate, whereas no effect was seen in the remaining three subjects. The test was repeated in these four subjects using a dose of 3 g. In two subjects (No. 12 and 13) there was a clear effect, and in two a doubtful effect that was possibly present 20 min. after stimulation. The latter two subjects were patients with peptic ulcer and hypersecretion.

For the purpose of observing the effect of sodium malonate on acid secretion under physiological conditions, 11 test subjects with acid gastric secretion received 2 g of sodium malonate 1 1/2 hrs after a normal meal consisting of bread, butter, fish or meat, and porridge and milk. Following the ingestion of sodium malonate, samples of the gastric contents were taken during 1—1 1/2 hrs.

Earlier experiments with a total of 68 test persons with acid gastric secretion have shown a mean gastric pH of 2 two hours after a meal of this kind (1, 2). It was slightly over 4 in one case only, between 3 and 4 in 9 cases, and below 3 in the remaining 58 cases.

Since the present 11 test subjects received 2 g of sodium malonate 1 1/2 hrs after the meal, the pH of the gastric content half an hour later was over 5 in 3 test subjects, 3—5 in 7 and lower or 2.5, in one subject only. The mean pH of the gastric content was then 4.0.

The mean pH 2 1/2 hrs after the meal was 3.8. In two subjects only it was below 3 (2.3 and 1.5).

At 3 hrs after the meal the gastric content, taken only from 7 subjects, had a mean pH of 4.4. It was less than 3 in only one test subject.

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Table I Effect of sodium malonate on the pH of gastric content after betazole hydrochloride (b h.) stimulation

Test sub-jects	Sodium malon-ate (g)	Before b h.	After b. h.		
			20	40	60
1	—	6.4	4.5	2.5	2.5
	2 0	6.6	6.0	5 4	3.5
2	—	4.8	2.0	2.0	1.5
	2.0	4 0	5.4	5.2	4.0
3	—	3.0	2.0	1.5	1.5
	2 0	2.5	5.4	4.5	3.0
4	—	2.0	1.5	1.5	1.2
	2.0	1.8	2.5	1.2	1.2
	3 0	2.0	4 4	1.5	1.2
5	—	4.5	2.5	2 0	2 0
	2.0	5.4	6.0	4.6	2.5
6	—	4.8	2.0	1.5	1.5
	2.0	2.8	6.6	7 0	6.8
7	—	1.5	1.5	1.2	2.0
	2.0	5 7	7 0	6.6	6 6
8	—	7.[illegible]	3.5	3.0	2.5
	2.0	7.2	7 0	7 0	6.6
9	—	5.2	3.0	2.3	1.5
	2 0	6 4	6 8	7 0	6.0
10	—	2.5	2.5	1.5	1.3
	2 0	5.2	5.0	4.2	2.0
11	—	6.6	—	1.5	1.5
	[illegible].0	2.2	2.5	3 0	2.5
	3 0	6.2	5.2	[illegible].5	2.0
12	—	4.[illegible]	2.0	1.5	1.5
	2.0	1 5	1.5	2.5	2.0
	3.0	6.6	6.4	5.0	1.2
13	—	1.5	1.2	1.5	1.2
	2.0	2.3	3 0	1 5	1.5
	3 0	4 6	5.2	3.8	1.5

Comparing the results with those obtained by Koskinen (2) under similar test conditions and using the usual antacids in doses equivalent with respect to neutralisation power it is seen that the results obtained with sodium malonate 2 and 2 1/2 hrs postprandially are, on the whole as good as those reached with sodium bicarbonate and magnesium oxide. Koskinen did not determine the pH at 3 hrs, but already 2 hrs 45 min. after the meal the gastric pH had begun to decline at least when sodium bicarbonate was used It therefore appears that the effect of sodium malonate on the acidity of the gastric content is probably of longer duration than that of the stated antacids Sodium bicarbonate and magnesium oxide of the antacids tested by Koskinen, had the strongest effect on the postprandial gastric acidity

Discussion

The presented tests have shown that sodium malonate clearly raises the pH of the gastric content under the described test conditions. The effect is based at least in part on the neutralisation power of sodium malonate. One gram of sodium malonate neutralises 56 ml of 0 1 N HCl to pH 3.5 In relation to the unit of weight its neutralisation power is about one half of that of sodium bicarbonate and sodium phosphate, and about one-eighth of that of magnesium oxide.

In view of the intensity and duration of the effect it appears possible that the effect of sodium malonate is based not solely on its neutralisation power Further studies on this subject are in progress.

Summary

Sodium malonate clearly raises the pH of the gastric content both after betazole hydrochloride stimulation and after a meal.

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A Study of Precipitating Antibodies against Human Intrinsic Factor

By

RAGNHILD GULLBERG and STEN KISTNER

Experimental results indicate that antibodies may be formed against Castle's intrinsic factor (I. F.) since antisera, obtained after immunization with preparations of this factor have been found to contain precipitating (1,2,3) and agglutinating (4) antibodies. Using gel diffusion technique Herbert and Kaplan (3) found evidence of several antigenic components in a preparation of purified hog I. F. and the antiserum also reacted with preparations of I. F. from other sources. Lowenstein et al. (4) used an antigen to wich radioactive vitamin B_{12} had been added. When reacting with the homologous antiserum a precipitate was formed but all radioactivity remained in the supernatant.

Since it has not been possible to obtain a pure antigen, precipitating antibodies against the I. F. have not been clearly demonstrated by *in vitro* techniques. In the present work an autoradiographic technique has been used to study the reactions between preparations of I. F. and their antisera.

Material and methods

Human gastric juice was collected according to an earlier described technique, including *in vivo* neutralization (5 6) from 4 healthy adult donors on 14 occasions. Vitamin B_{12}, containing a tracer dose labelled with Co^{60} was added in excess. After ultrafiltration, electrophoresis was performed at + 4 C. in polyvinyl chloride (7) for 20 hours at 4 V/cm, using sodium borate buffer of pH 9.0 μ 0.12. The more slowly migrating of the two B_{12}-binding fractions was eluated and concentrated by ultrafiltration. The total amount of the B_{12}-binding fraction isolated was found to contain about 50 μg of the added vitamin, as calculated from the amount of radioactivity recovered. About 4/5 of this preparation was used to immunize a rabbit which was given the antigen, mixed in Freund' adjuvant, in intramuscular injections at 10-day intervals. After 3 such injections dose without adjuvant was given and 8 days later blood was collected and serum separated.

Hog I. F. concentrate (WES 942) was used for immunization of another rabbit according

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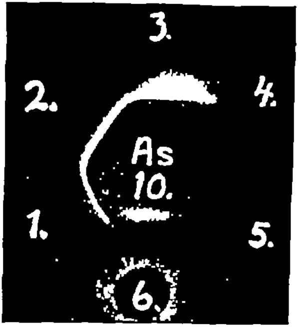


Fig 1 Autoradiogram of an agar gel diffusion experiment, showing the reaction between antiserum against human I F preparation, added in the central well (As 10) and different dilutions of ultrafiltered normal gastric juice, added in wells marked 1 2 and 3 and against hog I F preparation added in wells marked 5 and 6 Radioactive vitamin B_{12} had been added to these antigens. Normal human serum was added in the well marked 4

to the same principles. Each injection contained 25 mg

Antigen-antibody reactions were studied with agar gel diffusion technique (8) and immunoelectrophoresis (9) in sodium phosphate or veronal buffer at pH 6 7—8 6. The antisera were tested against ultrafiltered human gastric juice and hog I F concentrate, to which Co^{60} labelled vitamin B_{12} (specific activity about 1 $\mu C/\mu g$) had been added After the precipitin lines had developed, th gel was washed and dried and placed on Kodak Kodirex X ra film for autoradiography and then stained with amido schwartz.

Results

Several precipitin lines were observed when human gastric juice reacted with antiserum against the B_{12} binding fraction of human origin In the autoradiograms only one line appeared (fig 1 and 2) A similar result was obtained when the hog antigen reacted with its homologous antiserum.

When the hog antigen was tested with antiserum against the human I F preparation a faint precipitin line could be detected in the autoradiograms (fig 1 and 2) However complete fusion between this line and the line representing the reaction between the antiserum and the homologous antigen did not occur (fig 1) No reaction could be detected when human gastric juice was tested with antiserum against hog I F preparation (fig 2)

Immunoelectrophoresis combined with autoradiography showed that the line representing the reaction between the human B_{12} binding antigen and antiserum was located in the region of the β-globulins. The electrophoretic data indicated that the hog antigen was more acid than the human (fig 2) Other precipitin lines without signs of radioactivity were observed in the α_2- β- and γ areas, when human gastric juice reacted with antiserum against the human I F preparations. When the same antigen reacted with an antiserum against normal human serum no line could be detected with the autoradiographic technique

Discussion

The slowly migrating B_{12} binding electrophoretic fraction of human gastric juice has been shown to possess I F activity (10) It is the B_{12} binder deficient in gastric juice from patients with pernicious anaemia (6 11) Thus, this component was used for immunization. The results suggest that specific antibodies were formed against the I. F., since antibodies against a B_{12} -binding antigen were actually demonstrated This is in confor

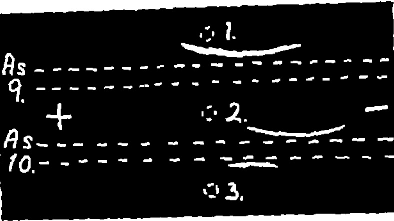


Fig. 2. Autoradiogram of immunoelectrophoresis at pH 6.7 of hog I. F. preparation (1 and 3) and ultrafiltered normal gastric juice (2). Antiserum against hog I. F. preparation (As 9), and against human I. F. preparation (As 10) were added to the long basins. All basins have been marked by dotted lines in the photographic reproduction.

mity with earlier observations on the in vitro inhibition of intrinsic factor by antiserum (1). In agreement with some earlier investigations (1,3) evidence of immunological cross-reactions between human and hog antigen was obtained, since the latter antigen to some extent reacted with anti-human I. F. serum. The reaction was very weak, compared with the reaction of this antiserum with the homologous antigen. The lack of complete fusion between the precipitin lines, representing the reaction with hog and human antigen respectively (fig. 1) shows that there was no identity between the B_{12}-binders. However, the cross-reaction indicates that both antigens have a certain similarity in structure. This lends further support to the idea that antibodies were formed against the I. F.

No reaction was observed between the anti-hog I. F. serum and human gastric juice. Lowenstein et al. (4) were not able to demonstrate cross-reactions in agglutination experiments with antiserum against hog preparation. Discrepant results concerning cross-reactions may be explained by differences in the antibody response and by variations in the antigenic character of I. F., which must be influenced by the procedure used for collection and preparation.

The technique described will be tried for the identification of B_{12}-binders in various tissues and secretions.

Summary

Antiserum was prepared against electrophoretically purified human intrinsic factor. Using gel diffusion technique and immunoelectrophoresis, several precipitin lines appeared when antiserum reacted with human gastric juice. Only one line was found in autoradiographs of such reactions, when radioactive vitamin B_{12} had been added to the antigen. The results indicate that specific antibodies were formed against the intrinsic factor.

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Studies on the Peripheral Circulation and Metabolism in Man

III. Oxygen Utilization and Lactate-pyruvate Formation in the Legs at Rest and during Exercise in Subjects with Hyperkinetic Circulation and Low Physical Working Capacity (Vasoregulatory Asthenia) and in Healthy Females

By

Lars A. Carlson Bengt Pernow and Staffan Zetterquist

In 1956 and 1957 Sjöstrand (1) and Holmgren et al. (2) described a group of patients with extremely low physical working capacity despite obviously normal cardiac and pulmonary function. This syndrome, which has recently been studied also by Tournaire et al. (3) is further characterized by an increased sympathetic tone, manifested as a pronounced orthostatic change in heart rate and typical ECG changes during and after work (3 4). Haemodynamic studies, including right-heart catheterization, showed normal intracardiac pressures and normal stroke volume, but a large cardiac output in relation to the oxygen uptake. The oxygen saturation of mixed venous blood was high at rest, and decreased less than normally during work. The low oxygen utilization was presumed to be due mainly to disorder of vasoregulation, with larger proportion of the cardiac output distributed through nonmuscular tissues than normally. An impairment of the transference of oxygen from blood to tissue could, however not be excluded (2). Physical training caused a remarkable increase in physical working capacity parallel to an increase of the central arterio-venous oxygen difference (5). The syndrome described was termed vasoregulatory asthenia (VA).

The VA cases showed a relatively moderate increase in arterial lactate concentration in relation to the heart rate during work. This increase was somewhat slower than in untrained men of the same age but faster than in well trained athletes (6).

In the present investigation the oxygen utilization as well as the lactate and pyruvate formation in exercising tissue have been studied by repeated withdrawal of arterial and femoral venous blood during leg exercise. In some cases similar studies were also performed in blood draining resting tissue during one leg exercise.

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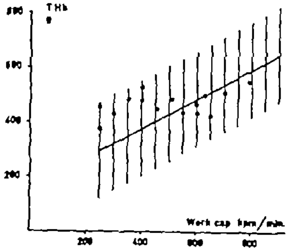


Fig 1 Total amount of haemoglobin (ordinate) in relation to physical working capacity (abscissa) Open circles denote VA cases, and filled circles female controls. *The straight line and hatched area indicate the normal regression line and* ± 2 standard deviation given by Holmgren et al. (2) Normal regression line equation $y = 0.545x + 160$ with a standard error of estimate for y (Sy) = 87.5 and coefficient of correlation (r) = 0.93.

cardiac and pulmonary pressures and normal stroke volume, but a large cardiac output in relation to the oxygen uptake as well as high oxygen saturation in mixed venous blood, both at rest and during work. When the data were plotted in the diagrams in ref. (2) these cases fell within the same group as the other VA cases.

Controls

The findings in the VA cases were compared with those in two control series. The first of these series consisted of 9 females aged 23—45 years. Five were nurses, one was a medical student and three were housewives. All were clinically healthy The physical working capacity ranged from 550—800 kpm/min (mean 600) and was in every case normal in relation to the amount of haemoglobin (fig 1). The ECG was normal both at rest and during exercise. The second control series has been described earlier (9) and consisted of 10 males aged 20—37 years and 5 aged 48—56 years.

Case material

Vasoregulatory asthenia (*VA*)

The case material consisted of 11 females, 6 of whom were included in the series of Holmgren et al. (VA 2 3 9 21 22 and 24 in refs. 2 and 4) At the time of the present study they were re-examined for orthostatic changes in heart rate, ECG at rest and during exercise physical working capacity total haemoglobin and heart volume. The methods used were the same as those previously described (9) In these cases, the working capacity — defined according to Sjöstrand (7) and Wahlund (8) as the work in kpm/min performed at a heart rate of 170 beats/min in a relatively steady state — was at the present examination 150—450 kpm/min (mean 350) during two-leg exercise. The 5 additional cases were also women, 20—47 years of age, with a physical working capacity of 200—350 kpm/min. All these values were lower than those predicted from the total amount of haemoglobin (fig 1)

Right heart catheterization in three of the latter cases revealed normal arterial, intra

Methods

A polyethylene catheter was *inserted per* cutaneously in one brachial artery (all cases), as well as in one or both femoral veins (8 VA cases and 5 controls) Exercise was performed as described in the first paper in this series (9) i. e., sitting on a bicycle ergometer with one leg working and the other resting The initial load was 150 kpm/min, and was increased by 150 *kpm/min every 5 minutes.* Exercise was continued until the heart rate had risen to above 160/min. Blood was withdrawn from the catheters simultaneously both at rest and at the end of every load. In four VA cases, femoral venous blood was drawn simultaneously from the exercising and resting legs. All values at rest refer to the supine position, and those during exercise to the sitting position. In two VA cases, studies of the peripheral circulation were also made in connection with right-heart catheterization, a two-leg exercise being performed in the supine position.

The methods for determination of oxygen saturation, pH, lactate and pyruvate concentrations in blood were identical with those used in a previous paper in this series (9)

Table I. Data obtained in metabolic studies of healthy females and VA cases

		Controls				VA			
		Rest	Exercise at heart rate			Rest	Exercise at heart rate		
			120	140	160		120	140	160
Venous oxygen saturation (%)	N	5	5	5	5	8	8	8	7
	M	69	30	26	24	87	43	41	40
	SD	13	4	4	4	9	6	10	7
Arterial lactate (mM/l)	N	9	9	9	9	11	11	11	11
	M	0.82	2.13	3.04	4.03	0.88	1.87	2.40	3.10
	SD	0.08	0.86	1.09	1.51	0.21	0.58	0.65	0.67
Venous lactate (mM/l)	N	5	5	5	5	8	8	8	8
	M	0.88	2.16	2.95	4.44	0.92	1.97	2.57	3.24
	SD	0.21	0.71	0.84	2.42	0.19	0.44	0.56	0.64
Arterial pyruvate (mM/l)	N	5	5	5	5	5	5	5	3
	M	0.10	0.12	0.14	0.17	0.10	0.11	0.13	0.16
	SD	0.01	0.01	0.02	0.02	0.01	0.02	0.03	0.03
Venous pyruvate (mM/l)	N	5	5	5	5	8	8	8	8
	M	0.10	0.21	0.25	0.24	0.09	0.17	0.20	0.23
	SD	0.03	0.02	0.03	0.03	0.02	0.02	0.03	0.02

N = number of observations. M = mean; SD = standard deviation.

Table II. Heart rate, central and peripheral arteriovenous oxygen difference (AV-O_2 diff.) and oxygen consumption (O_2 cons.) determined at rest and during supine exercise in two VA cases and two healthy subjects, all females of comparable age. Blood was simultaneously withdrawn from catheters in the brachial artery (BA), pulmonary artery (PA) and femoral vein (FV). The leg blood flow was roughly calculated with Fick's formula $\frac{O_{2R}}{AV - O_{2R}}$ in which O_{2R} denotes the regional oxygen uptake, and $AV - O_{2R}$ the regional arteriovenous oxygen difference. It was assumed in this calculation that, at rest, 15 % of the oxygen uptake in the lungs was utilized in the legs (21). For calculation of the leg blood flow during exercise it was assumed, that the whole increase in oxygen uptake during work was utilized in the exercising muscles.

Case	Kpm/min	Heart rate beats/min	AV — O diff. ml/l		O cons. ml/min	CD l/min	Calculated blood flow (l/min) at rest and during exercise	
			BA — PA	BA — FV			Leg	Other tissues
VA 1	Rest	98	24.6	12.5	214	8.7	3	6
	300	164	67.1	106.3	1,006	15.0	8	7
VA 2	Rest	102	26.7	18.3	210	7.9	2	6
	150	170	73.1	121.2	902	12.6	7	6
N 1	Rest	74	32.8	33.8	247	7.5	1	6
	300	115	77.8	113.9	882	11.5	6	5
	600	180	134.9	160.9	1,987	14.7	11	4
N 2	Rest	62	35.5	34.2	219	6.1	1	5
	600	158	116.9	149.7	1,439	12.5	9	3

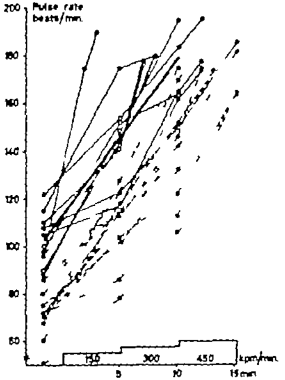


Fig. 2. Heart rate (beats/min) at rest and during one-leg exercise in relation to absolute work load (kpm/min) o —— o = VA cases — — = female controls. The thick lines represent the regression lines of the respective groups. Regression line equation for VA y = 0.24x + 103.02, Sy = 18.2 r = 0.86. Same equation for the healthy females y = 0.22x + 72.7 Sy = 19.7 r = 0.89.

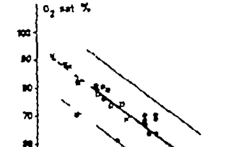


Fig. 3. Femoral venous oxygen saturation ( ) at rest correlated to heart rate (beats/min) at rest. o = healthy males (8) □ = healthy females = patients with arteriosclerosis obliterans (14) = VA cases.

Results

The results are collected in tables I—II and figs. 2—6.

The heart rate in the healthy females was 48—98 beats/min at rest. During exercise — when one subject reached a maximal load of only 300 kpm/min, whereas 7 of the others achieved 450 kpm/min and one 600 kpm/min — a steady rise in heart rate to 162—182 beats/min was recorded.

In the VA cases, the resting heart rate was significantly higher than in the healthy subjects (72—122 beats/min, mean 103 $p < 0.001$). During one leg exercise as well, the heart rate at each load was significantly higher in the VA cases ($p < 0.001$). However the regression lines for the heart rate during stepwise increasing work loads did not differ significantly from each other with regard to the slope the regression coefficient being 0.24 and 0.22 respectively (fig. 2).

The oxygen saturation of the *arterial* blood in both the female controls and the VA cases was within the normal range at rest (96—99 %) and was unchanged during exercise.

The oxygen saturation of the femoral *venous* blood in the controls was 47—79 % (mean 69) at rest, which was within the normal range in the previous series of healthy males. During exercise, a small but successive fall in femoral oxygen saturation from the lowest load to the highest was observed in all cases. The lowest values, 22—29 (mean 24) were recorded at a heart rate of 160—185 beats/min. When plotted against the increase in heart rate during work, all the values for oxygen saturation of the femoral venous blood were within the normal range found in healthy males (fig. 4).

In the VA cases, the oxygen saturation of the femoral venous blood was 68—95% (mean 78) at rest, i. e., significantly

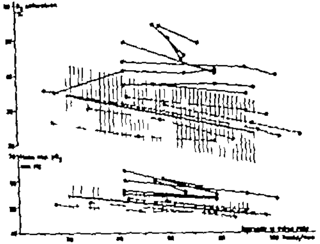


Fig. 4. Femoral venous oxygen saturation in % (upper diagram) and calculated mean capillary pO in mm Hg (lower diagram) during one-leg exercise. The values are plotted against the increase in heart rate from the resting value.
—— = VA cases; – – – = female controls. Thick lines represent the normal regression lines of the control male series (9). Equation of upper diagram $y = 55.35 - 0.138x$, $s_y = 4.4$, $r = -0.56$. Equation of lower diagram $y = 33.42 - 0.058x$, $s_y = 2.7$, $r = -0.44$. Hatched areas show variations in $\pm$ 2 standard deviation.

higher than in the controls ($p < 0.001$). It is evident from fig. 5 however that the venous oxygen values for the VA cases fit into the inverse relationship between heart rate and oxygen saturation found for normals. At the first work load the venous oxygen saturation of the exercising leg was 35—55 % (mean 45) and decreased slightly during continued work. At the highest load, a mean saturation of 42 % was recorded at a heart rate of 160—185 beats/min, which was significantly higher ($p < 0.001$) than in both healthy females and males (table I and fig. 4).

The mean capillary pO_2 calculated with the formula of Barcroft (10)

$$\text{venous pO} + \frac{\text{arterial pO} - \text{venous pO}}{3}$$

in the healthy females was 37 mm Hg (mean value) at rest. This value, as well as the decrease in pO during work, was completely within the normal range of the male subjects (fig. 4). In the VA cases, a mean resting value of 32 mm Hg was calculated. Also during work the mean capillary pO was in general higher in VA than in the normals. In both healthy females and VA cases the decrease in mean capillary pO was well correlated with the decrease in venous oxygen saturation, since no significant changes were observed in the arterial pO_2.

In four VA cases doing one-leg exercise, the venous oxygen saturation was studied in the resting and the exercising leg simultaneously. Normally this value has been found to fall to the same degree in the exercising and the resting leg, the drop sometimes being even more pronounced in the latter, especially at high heart rates (11). The VA cases, in contrast, showed a tendency in the opposite direction, i. e., during one-leg exercise, the venous oxygen content tended to be higher in the resting leg, particularly at high heart rates (fig. 5).

The lactate concentration at rest in the healthy females was 0.73—0.95 mM/l (mean 0.88) in arterial blood, and 0.65—1.09 mM/l (mean 0.92) in femoral venous blood. This difference was not significant. In figs. 6—8, the increase in arterial and venous lactate during work is plotted against the absolute and relative work intensities. At the highest loads (heart

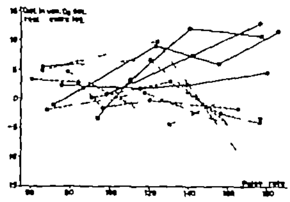


Fig 5 Difference in femoral venous oxygen saturation (%) between resting and exercising leg during one-leg exercise. o —— o = VA cases — — = healthy subjects.

rate 160—185 beats/min) the arterial lactate ranged in 11 cases from 2 71 to 8 40 mM/l (mean 4 73) Corresponding values for femoral venous lactate in 5 cases were 3.21—6 42 mM/l (mean 4 62) In all but two cases the increase in lactate during work was much slower in the females than in the healthy males when relating lactate to the absolute heart rate during work (fig 6) However when relating lactate to the increase in heart rate from the resting level during work, all females fell within the ± 2 standard deviation of the regression line of the males. However in this relation, the *mean* values in the females at the different heart rates were significantly lower ($p < 0.01$) than in the males (fig 7)

In the VA cases, the lactate concentration at rest was 0 72—1 12 mM/l (mean 0 88) in arterial blood and 0 77—1.21 mM/l (mean 0.92) in femoral venous blood which did not differ significantly from the values in healthy females. During work, there was a significantly *more rapid increase in arterial lactate in* VA than in the healthy females when related to the absolute work ($p < 0.001$ at 300 kpm/min fig 8) or to the increase in heart rate during work ($p < 0.005$ fig 7) No difference was, however found when relating the arterial lactate to the absolute heart rate during work (fig 6) The same tendency was observed on the venous side, even if this series was too small to permit a statistical analysis (fig 9) At a heart rate of 160—185 beats/min, the mean lactate concentration was 4 05 mM/l in arterial blood and 4 44 mM/l in femoral venous blood, this difference being significant ($p < 0.01$)

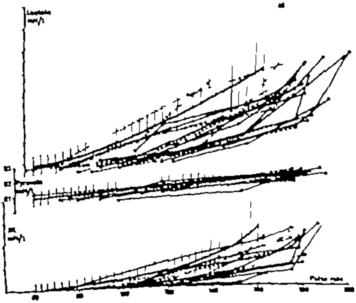


Fig 6. Arterial lactate pyruvate and "excess of lactat (XL) in mM/l at rest and during one-leg exercise in VA (o —— o) and female controls (— —) The exercise values are plotted against the heart rate during work. Thick lines and hatched areas *indicate mean values and variations* in ± 2 standard deviations of control male series.

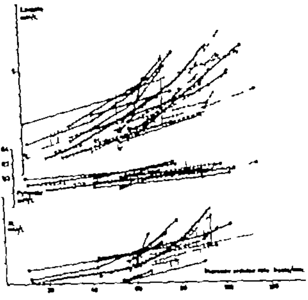


Fig. 7 Arterial lactate, pyruvate and XL (mM/l) during exercise in relation to increase in heart rate during work. Symbols as in fig. 6. Thick lines and hatched areas indicate mean values and variations in ± 2 standard deviations of control male series.

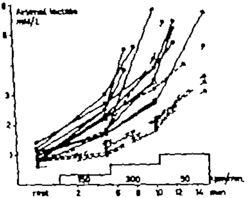


Fig. 8. Arterial lactate (mM/l) at rest and during one-leg exercise in relation to absolute work intensity (kpm/min). Symbols as in fig. 6

The *pyruvate concentration* at rest in the healthy females was 0.09—0.11 mM/l (mean 0.10) in arterial blood, and 0.08—0.13 mM/l (mean 0.10) in femoral venous blood. During exercise, the pyruvate increased successively and at the highest load (heart rate 160—185) reached 0.18—0.29 mM/l (mean 0.22) in arterial blood, the corresponding value for the venous lactate being 0.18—0.30 mM/l (mean 0.27). This increase was smaller than in the healthy males when related to the absolute heart rate during work, but normal in relation to the increase in heart rate from the resting level during work.

In the VA cases, the resting values for pyruvate were 0.09—0.11 mM/l (mean 0.10) in arterial blood, and 0.07—0.11 mM/l (mean 0.09) in femoral venous blood. Thus, no significant difference was present between the resting values in VA cases and in healthy females ($p < 0.5$). At heart rate of 160—185 beats/min, the arterial pyruvate in the VA cases ranged from 0.18 to 0.29 mM/l (mean 0.22) the corresponding value in femoral venous blood being 0.23—0.30 mM/l (mean 0.25). In most VA cases, the increase in arterial pyruvate was more rapid than in healthy females in relation to the increase in heart rate from the resting level (fig. 7) whereas no difference was observed when the increase was related

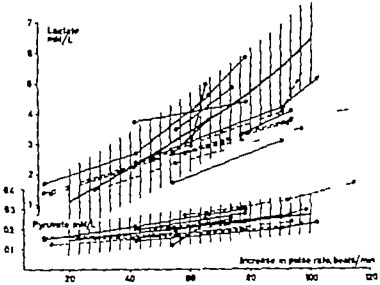


Fig 9 Femoral venous lactate and pyruvate (mM/l) at rest and during one-leg exercise in VA (o —— o) and female controls (——) The exercise values are plotted against the heart rate during work. Drawn lines and hatched areas indicate mean values and variations in ± 2 standard deviations of control male series.

to the absolute heart rate during exercise (fig 6) The same tendency was noted for femoral venous pyruvate.

The "*excess of lactate* (XL) was calculated from the formula

$$XL = (L_n - L_o) - (P - P_o)\left(\frac{L_o}{P}\right)$$

where L_o and L_n represent the lactate concentration at rest and at different loads, and P and P_n the corresponding values for pyruvate (12) No difference was found between healthy females and males with respect to the increase in XL during exercise, when the data were related to the rise in heart rate during work. In VA, on the other hand, there was a significantly more rapid increase in XL during work than in healthy females ($p < 0.05$) (fig 7)

Discussion

The results obtained have mostly been related to the relative work load expressed as heart rate during work. The various differences between VA and controls become in all respects greater when related to the absolute work, but as the type of exercise performed is mainly limited by the oxygen transporting capacity it seems most adequate to compare the different cases at a degree of work, at which the load on the central circulation is similar

In the original communication on patients with vasoregulatory asthenia, it was supposed that the low physical working capacity was due to inadequate regulation of the peripheral blood flow at rest and during work (2) The abnormally high oxygen saturation in mixed venous blood during work was taken as evidence of an abnormal distribution of the blood flow with a larger portion draining nonmuscular tissues than in normal subjects. This dysregulation of the peripheral blood flow could be adjusted by physical training (5)

It has been shown in the present study that the oxygen saturation of venous blood from the exercising leg decreased much less during exercise in the VA cases than in healthy males and females. Similar observations have recently been made in patients with myoglobinuria and extremely low physical working capacity (13) This high venous oxygen saturation might be due to 1) an abnormal

distribution of the blood flow between muscular and non-muscular tissue 2) arteriovenous shunting of blood or 3) an oxygen diffusion block.

ad 1) The catheter was inserted 10 cm distally into the femoral vein, just below the inguinal ligament. With this technique the catheter tip was probably distal to the entry of the long saphenous vein, which drains most of the superficial non-muscular tissues of the thigh and calf. Even at this level is, however the deep vein system supplied with cutaneous blood by the low entry of the short saphenous vein. Consequently the blood drawn at rest originates from both muscular and non-muscular tissue. During exercise, however a redistribution of the blood is supposed to occur normally with the major portion flowing through the exercising muscles. This is particularly evident in patients with impaired peripheral circulation due to arteriosclerosis obliterans, where in some cases an almost complete extraction of the available oxygen from the blood has been recorded (14) Even the change in position from supine to sitting obviously causes marked changes in blood flow and possibly also in blood distribution from non-muscular to muscular tissues, since such a positional change is accompanied by a pronounced decrease in oxygen saturation of the femoral venous blood (9 15) This was also observed in one of the VA cases the femoral venous oxygen saturation decreased from 80 to 37 % merely by changing the position from supine to sitting During exercise in the sitting position, the value initially rose to 49 %, and thereafter fell successively to 40 during continued work. Such a rise in venous oxygen saturation from the resting level during exercise was never observed in the controls and was evidently due to a redistribution of blood from muscular to non-muscular tissues, or to arteriovenous shunting of blood in the muscles.

The disordered distribution of blood between resting and exercising tissues in VA is further illustrated in fig 5 which shows, that in three of four VA cases the decrease in femoral venous oxygen saturation was less in the resting leg than in the exercising one, while normally the venous oxygen saturation was found to decrease to the same extent or sometimes even more in the resting leg than in the exercising, evidently due to a restriction of the blood flow through resting tissues (11) This must indicate, that in VA a larger proportion of the total blood flow is distributed to resting tissues during exercise than normally This finding is in conformity with the observation of Graf and Ström (16) who by a plethysmographic technique recorded a greater blood flow through the forearm during leg exercise in VA cases than in normal subjects.

ad 2) The existence of arteriovenous anastomoses has recently been demonstrated in man by the microarteriographic technique (17) It was observed that the arterial and venous systems were combined not only by capillaries, but normally also by bridges allowing flow of arterial blood directly to the veins. Indirect evidence of a shunt mechanism in skeletal muscle of the cat has recently been presented by Hyman et al. (18) and Rosell and Uvnäs (19) Hyman et al (18) suggested that non-nutritional vessels were opened up by cholinergic vasodilator nerves.

ad 3) An impaired delivery of oxygen from the blood to the tissue as a cause of the low oxygen utilization has been discussed earlier (2) Hypothetically the diffusion block might either be local in

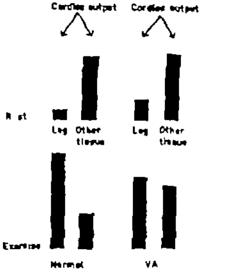


Fig 10 Schematic presentation of the distribution of the cardiac output between the legs and other tissues at rest and during leg exercise in healthy subjects and VA cases. The diagram is based on mean values from table II

the exercising muscles or due to a general metabolic disturbance. No studies have sa far been made of the peripheral oxygen diffusion in these cases The Hb—HbO dissociation curve was determined in two cases, and found to be normal (2)

It has earlier been suggested that the most likely explanation of the VA syndrome is a disorded distribution of the cardiac output (2) The data presented in this paper seem to support this hypothesis. The difference between VA and the normals in the distribution of the cardiac output is illustrated schematically in fig 10 based on the calculations given in table II At rest, the cardiac output is greater in VA than in normals and it is evident from the values for arteriovenous oxygen difference over the leg (BA—FV) in comparison with the total arteriovenous difference (BA—PA) that the greater blood flow in VA is specially distributed to the legs. This is in agreement with the observation that in VA the resting blood flow in skeletal muscle is about twice the normal (16) During work the cardiac output increased more in the VA cases than in the normal subjects at comparable absolute work intensities. The restriction of the flow through resting tissues, which normally occurs during exercise, was, however much less pronounced in VA, resulting in a smaller than normal increase in the blood flow through the exercising tissue. As is seen in table II and fig 10 about 75 % of the cardiac output normally flows through the exercising legs whereas the corresponding values in the VA cases is only about 50 % at comparable heart rates.

A significantly more rapid increase in lactate during exercise was observed in the VA cases than in the healthy females, when the data were related to the absolute work intensity or to the increase in heart rate from the resting level during work (fig 7—8) This is in agreement with the findings by Linko (20) who showed that patients with neurocirculatory asthenia with low physical working capacity had a higher lactic acid concentration of the blood than healthy subjects of the same profession at comparable work loads. This evidently implies a greater anaerobic component of the total metabolism during work, which is probably a consequence of the above mentioned fact that a smaller part of the cardiac output is distributed to the exercising tissues in VA than normally (fig 10)

Summary

1 The utilization of oxygen and formation of lactate and pyruvate during exercise has been studied in 11 cases of vasoregulatory asthenia (2) The results are compared with those obtained in 9 healthy females, as well as in a previous series of 15 male controls (9)

2. The oxygen saturation of the femoral venous blood at rest is significantly higher in the VA cases than in the healthy females. In the latter group, the fall in this value during exercise is found to be within the normal range for healthy males, whereas it is significantly less in the VA cases. In resting tissue, the decrease in venous oxygen saturation is less than in the controls, evidently indicating that a larger proportion of the total blood flow is distributed to resting tissues during exercise in the VA cases than in the normals.

3. During exercise the blood lactate and pyruvate are found to increase more rapidly in the VA cases than in the healthy females, when related to the absolute work intensity or to the increase in heart rate from the resting level. The increase in lactate and pyruvate during exercise in relation to heart rate is significantly slower in all females than in the healthy males.

4. The results obtained seem to give further support to the earlier suggestion that the low physical working capacity in the VA cases is due mainly to an abnormal distribution of the blood flow during exercise with a smaller part of the cardiac output flowing through the exercising muscles than in the normals.

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Serum Cholesterol and Heredity

A Twin Study

By

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The purpose of the present study is to contribute to the discussion of the relative importance of heredity and environment for serum cholesterol.

According to Wilkinson *et al.* (1) idiopathic hypercholesterolaemia may be caused by a dominant gene, but with incomplete penetrance. However the work of Schaefer et al. (2) has shown that genetic factors are of importance, even where serum cholesterol is on the normal level. Osborne et al (3) in a twin study found larger intra-pair variances for dizygotic than for monozygotic twins, although the variance ratios were not statistically significant.

Material and methods

The twins for this study were collected through the public schools in Oslo. Some pairs were discovered through chains of acquaintances. All the pairs, except two, were living in Oslo.

Twins have been counted as *monozygotic* if they were so alike in features that one might easily be mistaken for the other, if they showed complete concordance in blood types (AB0, MN, Rh, and Kell systems) and considerable similarity with respect to finger prints, and if information available about the birth did not make monozygosity unlikely.

Twins have been counted as *dizygotic* if they were distinctly unlike in features, and if available information about the birth fitted with dizygosity.

Three pairs which were initially examined have been excluded for the following reasons: In two pairs the information about the birth was incompatible with monozygosity, whereas the other information pointed towards monozygotic twins. In one pair the similarity of features was not complete, although serology and finger prints pointed towards monozygosity.

Included in the material is one set of monozygotic triplets.

All were healthy at time of examination, and there was no serious disease in the history. In most cases blood was taken simultaneously from the twins in a pair. In the other cases the blood was taken at the same time of the day. None had been fasting in advance of the test.

Serum total cholesterol was determined at the Johan Throne Holst Institut for Nutritional Research by Hauge and Nicolaysen method (4). This method has been internationally tested (5).

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Table I Summary of material

Age	No. of pairs of twins					
	Mono-zygotic		Dizygotic			
			Like sex		Different sex	
	Living together	Living apart	Living together	Living apart	Living together	Living apart
10–19	19	—	11	—	12	—
20–29	1	—	—	—	—	—
30–39	—	2	1	—	—	—
40–49	—	3	2	3	—	—
50–59	—	1	—	—	—	—
All ages	*20*	*6*	*14*	*3*	*12*	*0*

One set of triplets is counted as two pairs.

Table I provides a summary of the age distribution, gentic composition and residence characteristics in the material. Table II specifies age sex and cholesterol for each of the included individuals.

In a study like the present one it is the difference between twins which is of interest, rather than the absolute level of cholesterol. The *intra-pair variance* has been used for comparing the cholesterol differences in the various types of twin pairs. The same statistic was used by Osborne et al (3)

Results

The genetic influence on cholesterol level is illustrated in table III where monozygotics living together are compared with dizygotics *of same sex living* together Neither in the material by Osborne et al. (3) nor in the present material were there any great differences between male and female variances. The two sexes have therefore been combined

It will be seen that the present material has given a sharper contrast between the two types of twins. The variance ratio is 4 91 which is highly significant It may

Table II Genetic composition, residence status, sex, age and serum cholesterol for included twin pairs

Age	Sex	Choles-terol	Age	Sex	Choles-terol	Age	Sex	Choles-terol
Monozygotic, living together								
13	F	139 146	14	M	190 163	14	F	174 178
14	M	150 159	14	M	202 190	15	M	190 210
15	F	178 216	16	F	199 225	16	M	154 155
16	F	182 174	16	F	163 187	16	F	202 220
17	M	121 132	17	M	168 193	17	M	165 194
17	M	125 134	18	M	199 200	19	M	145 162 163
21	F	208 216						
Monozygotic, living apart								
31	F	204 259	36	F	288 239	41	F	493 485
41	M	244 248	41	M	300 280	56	M	232 279
Dizygotic same sex, living together								
14	F	155 179	14	F	177 185	15	M	175 152
15	M	127 175	15	M	181 189	15	M	138 194
16	M	210 177	16	F	296 220	16	F	149 159
18	F	214 171	18	M	187 175	31	M	184 212
41	M	220 240	42	M	268 205			
Dizygotic, same sex, living apart								
41	F	282 336	43	F	234 220	45	F	304 342

A triplet, male, was not identical with the tw monozygotic sibs. He has been excluded from the present material (cholesterol 148).

Table II (cont.)

Age	Sex	Cholesterol	Age	Sex	Cholesterol	Age	Sex	Cholesterol
Dizygotic, different sex, living together								
14	M	165	14	M	157	14	M	131
	F	165		F	205		F	226
14	M	167	15	M	147	15	M	199
	F	137		F	187		F	169
16	M	126	16	M	140	16	M	114
	F	212		F	135		F	156
17	M	167	17	M	134	18	M	179
	F	181		F	171		F	180
Dizygotic, different sex, living apart								
None examined								

Table III Intra-pair variance of serum cholesterol in monozygotic twins living together and dizygotic twins of like sex living together

	Present material		Osborne et al. (3)	
	No. of pairs	Intra-pair variance	No. of pairs	Intra-pair variance
Dizygotic, same sex	14	742	15	497
Monozygotic	20	151	28	243

One set of triplets is counted as two pairs.

be mentioned that the variance ratio for males was 5.8 and for females 4.2.

Table IV compares monozygotic twins, living together with monozygotic twins living apart. Thus, the table illustrates environmental influences. It should be noted in this connection that scrutiny of the material revealed no association between age and intra-pair difference.

Table IV Intra-pair variance of serum cholesterol in monozygotic twins living together and monozygotic twins living apart

	Present material		Osborne et al. (3)	
	No. of pairs	Intra-pair variance	No. of pairs	Intra-pair variance
Living apart	6	676	15	515
Living together	20	151	28	243

One set of triplets is counted as two pairs.

Table V Intra-pair variance of serum cholesterol in dizygotic twins living together. Pairs of like sex compared with pairs of different sex

	Present material		Osborne et al. (3)	
	No. of pairs	Intra-pair variance	No. of pairs	Intra-pair variance
Different sex	12	1,228	5	503
Same sex	14	742	15	497

The variance ratio in the present material is 4.48, which is highly significant. It may be noted that the 6 pairs living apart includes one pair with hypercholesterolaemia. If this pair is excluded the ratio becomes 3.33.

Table V includes dizygotic twins, living together. Pairs of like sex are compared with pairs of different sex. The variance ratio 1.65 is not significant.

It may be noted that the variance for pairs of different sex, 1,228 is less than half the variance of 2,630 that was found among 5 454 healthy males in Oslo age 40—59 (6).

Summary

The present study of serum cholesterol in twins showed

a) Significantly higher differences in dizygotic twins of same sex living together than in monozygotic twins living together

b) Significantly higher differences in monozygotic twins living apart than in monozygotic twins living together and

c) Slightly (non-significantly) higher differences in dizygotic twins of different sex living together than in dizygotic twins of same sex living together

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From Medical Department A (Head K. Brøchner Mortensen, M.D.)
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Glucose Tolerance in Acute Ischemic Renal Failure

By

Uffe Sagild

In 1917 Hamman and Hirschmann (8) reported that some patients with chronic uremia showed a decreased glucose tolerance, as determined by a standard oral glucose tolerance test. This observation was later confirmed by other workers (11). Hyperglycemia during fasting has also been noted occasionally in azotemic patients (12).

The possible defects in carbohydrate metabolism in uremia have only been poorly delineated, but recently Perkoff et al. (13) suggested that urea may have a specific effect on glucose metabolism in reducing the rate of glucose assimilation.

With the exception of some experiments on urea loading in normal subjects, reported in the paper by Perkoff et al. (13) observations on carbohydrate metabolism in human uremia have been made almost entirely in groups of patients with chronic renal disease and the conclusions drawn from comparison with data obtained in normal subjects. This comparison is difficult, because among other things the normal range of variation in the response to an oral or intravenous glucose load is rather wide, and also because chronic renal disease frequently is complicated by electrolyte disturbances, which may in themselves affect the glucose metabolism (14).

In the present work the problem has been approached through a study of glucose metabolism in patients with acute ischemic renal failure, but without previous renal disease. In each patient studied several determinations of the glucose assimilation were performed. During the period of the study large fluctuations in the degree of azotemia occurred in each patient, while at the same time major electrolyte disturbances could be controlled. Thus, observations on glucose metabolism could be correlated with varying degrees of azotemia in the same patient.

Material and methods

Subjects

The patients studied were five females and four males admitted to the dialyzing unit of the medical department A, Rigshospitalet,

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Summary

The present study of serum cholesterol in twins showed

a) Significantly higher differences in dizygotic twins of same sex living together than in monozygotic twins living together

b) Significantly higher differences in monozygotic twins living apart than in monozygotic twins living together and

c) Slightly (non-significantly) higher differences in dizygotic twins of different sex living together than in dizygotic twins of same sex living together

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With the exception of some experiments on urea loading in normal subjects, reported in the paper by Perkoff et al. (13) observations on carbohydrate metabolism in human uremia have been made almost entirely in groups of patients with chronic renal disease and the conclusions drawn from comparison with data obtained in normal subjects. This comparison is difficult, because among other things the normal range of variation in the response to an oral or intravenous glucose load is rather wide, and also because chronic renal disease frequently is complicated by electrolyte disturbances, which may in themselves affect the glucose metabolism (14).

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Three of the patients died in the hospital The causes of death were severe hypotension with convulsions (no. 3) cardiac failure (no 9) and overwhelming infection (no. 1) In all cases death occurred several days after the completion of this study The remaining patients all survived without evidence of permanent renal damage.

In a control group of ten patients, the reliability of the test procedure (see below) was checked by duplicate determinations. Five of these had mild diabetes, while the remaining five had other chronic diseases without known impairment of carbohydrate metabolism. The interval between tests in this group varied from three to seven days.

In the dialyzing unit the patients were treated according to the principles outlined in a previous communication from this department (6) This implies, in general terms

During the "retention phase" no protein is allowed and fluids are restricted to permit daily weight losses of the patient ranging from 300 to 500 g/24 hours. Supplementary hemodialysis is generally carried out whenever the blood urea concentration (BU) exceeds 400 mg % During the present study hemodialysis was performed once in each of three patients (no. 1 4 and 6)

During the "excretory phase" urinary and extrarenal losses of water and electrolytes are quantitatively replaced, and the patients are offered an unlimited diet with as much protein and glucose as they can tolerate.

This treatment necessitates daily estimation of the patient's weight and intake and output of water and electrolytes (sodium and potassium) In addition, the concentrations of hemoglobin, total protein, blood urea, serum sodium, potassium, -chloride, bicarbonate and -creatinine are estimated daily

Test procedure

During four to six hours prior to each test no caloric intake was allowed perorally or by vein. After a capillary blood sample had been obtained from the lobe of the patient's ear with minimal or no squeezing exactly 50 ml of a 45 % w/v solution of glucose in water were injected within three minutes through a needle in an antecubital vein For the injection the system proposed by Barnett & Fellers (2) was used. This permitted an accurate assessment of the amount of glucose injected. Subsequently capillary blood specimens were obtained from the ear lobe at five to seven minute intervals for sixty minutes.

From two to eight tests were performed in each of the patients with acute renal failure, making a total of thirty-three tests. No tests were performed in patients with fever or muscular unrest.

Chemical methods

The blood samples for glucose determination were pipetted directly into freshly prepared zinc hydroxide and analyzed in duplicate immediately following the test according to the Hagedorn-Norman Jensen method (7). Sodium and potassium in urine and blood were determined by flame photometry urea by the method described by Conway (4) and creatinine according to Bonnes & Tausky (3)

Data analysis

In accordance with Amatuzio et al. (1) and Duncan (5) it was found that the blood glucose value in excess of the initial value (increment blood glucose) fell exponentially with time, so that when logarithm of the increment blood glucose was plotted against time, a straight line resulted. By applying the method of least squares, the line representing the best fit of the points obtained, was calculated

An index of glucose tolerance was derived by applying the formula

$$K = \frac{\log 2}{t}$$

where t is the time in minutes required for the blood glucose increment value at any point to be halved. This index measures glucose tolerance as the fall of blood glucose concentration per minute in per cent of the increment blood glucose value at that time.

Table I. Clinical information on 9 patients with acute ischemic renal failure

No.	Sex	Age (yrs)	Primary disease	Creat. clear < 5 ml min (days)	Final out come	No. of gluc. tol. tests	Sequences			
							Retent. phase	Excretory phase	Hemo-dialysis	Total
1	F	72	Biliary occlusion, cholecystectomy	19	Died	4	2	–	1	3
2	F	59	Biliary occl. not op.	12	Surv	2	–	1	–	1
3	F	55	Traffic acc. fract. brain concussion	12	Died	2	1	–	–	1
4	F	71	Resection of bladder tumor	15	Surv	5	2	1	1	4
5	F	52	Op. for incarc. umbil. hernia	10	Surv	2	1	–	–	1
6	M	64	Resection of stomach	16	Surv	8	3	3	1	7
7	M	67	Biliary occlusion, cholecystectomy	8	Surv	3	1	1	–	2
8	M	73	Prostatectomy	6	Surv	3	–	2	–	2
9	M	77	Op. for strang. ileus	14	Died	4	2	1		3
					Total	33	12	9	3	24

An estimate of the volume of distribution of glucose (VDo) was obtained by applying the formula

$$VDo = \frac{N \times 10}{(Ao - FBS) \cdot 1.25} \text{ liters}$$

where N is the total amount of glucose injected (in mg), Ao is the theoretical glucose concentration (in mg%) at zero time obtained by extrapolation of the curve to zero time, and FBS is the fasting blood sugar (in mg%). The factor 1.25 in the denominator corrects for the solid content of whole blood, since the Hagedorn-Norman Jensen method employs whole blood.

In each patient K and VDo were estimated on different levels of azotemia, as determined by blood urea concentration (BU) and serum creatinine concentration. The changes in K and VDo relative to the changes in BU could be observed during a total of 24 sequences. As appears from table I uremia was aggravated in the retention phase during 12 sequences, reduced in the excretory phase during 9 sequences and reduced through hemodialysis during 3 sequences.

While the method of the study safeguarded against major changes in the patient total electrolyte pool during the retention and excretory phases, this could not be excluded during hemodialysis, because no measurements of net electrolyte transfers during this process were attempted.

Since the number of tests performed in each patient was arbitrary, statistical analysis of the accumulated data was not feasible.

Results

Reproducibility of test procedure

Duplicate intravenous glucose tolerance tests were performed in ten hospitalized subjects. The coefficient of variation

because of acute ischemic renal failure. Clinical information on the patients is presented in table I It appears that in seven instances an operation had preceded the development of acute anuria. Only in four of these had an episode of shock or prolonged hypotension been observed. In the remaining two of the nine patients, anuria was preceded by an attack of biliary colic with jaundice in one instance and a traffic accident with fractures of the pelvis in the other None of the patients had a history of previous renal disease or diabetes mellitus.

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of normal, as reported by Amatuzio et al. (1) (range 2.97—4.83) and Duncan (5) (range 3.15—4.62)

Inspection of the data further reveal, that although the K values varied considerably between individuals, there was a definite tendency to lower K-values with higher degrees of azotemia, as measured by the concentration of blood urea and serum creatinine.

Thus, in twelve sequences during the retention phase, increasing BU was associated with decreasing K in eleven instances, but with increasing K in one. In nine sequences during the excretory phase, decreasing BU (due to spontaneous diuresis) was associated with increasing K in seven instances, while in two the reverse was found. In three sequences hemodialysis was followed by increased K-values in all instances.

Thus, in 21 or 24 sequences, increments of BU were associated with decrements of K or vice versa. In three of these 21 sequences the observed percentage differences in K did not exceed the coefficient of variation of duplicate determinations of K in the control group. However in 11 sequences the percentage differences were more than twice this coefficient.

The serum potassium concentrations at the time of the tests are indicated in table II. They were usually within normal limits and the changes in glucose tolerance (as measured by K) could not be correlated with fluctuations in the serum potassium concentration. Similar observations were made as regards the serum sodium and the standard bicarbonate concentrations (not indicated in the table)

Volume of distribution of glucose (VD_G)

The changes in VD_G during the 24 sequences studied did not appear to be related to the changes in K or degree of azotemia, as measured by BU or serum creatinine. In most instances the changes in VD_G corresponded in magnitude and direction to the changes in total body water as judged by the day-to-day fluctuations in the body weight. Thus, the VD_G per kilo weight was found to remain rather constant in individual patients during large fluctuations in BU. The variation in VD_G kg body weight between the patients was considerable.

Discussion

Difference of opinion exists as to whether increment blood glucose values or the total glucose concentration in the blood should be used as a basis for the calculation of the slope of the intravenous glucose tolerance curve.

According to Amatuzio et al. (1) and Duncan (5) the former method is preferable, because it yields the best reproducible results and because the slope of the resulting curve is largely independent of the amount of glucose administered.

However this view has been opposed by Ikkos & Luft (10) and others, who believe, that the rate of disappearance of glucose should be calculated on the basis of absolute glucose value among other reasons because there is no evidence to suggest that the cells can differentiate between exogenous and endogenous glucose. This discussion can hardly be said to be finally settled as yet.

In the present work the Hagedorn-Norman Jensen method, which measures the total quantity of reducing substances in the blood, has been used for the determination of blood glucose, because this method in our hands yields more reproducible results than newer methods

Table II Main data from 33 intravenous glucose tolerance tests in 9 patients with acute ischemic renal failure

No.	K values	VDc (l)	VDc/W (l/kg)	Potassium (mEq/l)	Urea (mg%)	Creat. (mg%)	VDc (l)	Weight (kg)	Sequences	Interval (days)
1	1.86	10.2	0.18	4.1	220	6.4				
	1.59	9.6	0.17	5.9	360	9.8	− 0.6	− 1.3	R	3
	1.71	10.7	0.19	3.8	122	5.5	+ 1.1	+ 0.8	H	1
	1.46	10.2	0.18	3.0	266	7.5	− 0.5	− 1.0	R	7
2	3.22	15.9	0.17	4.2	150	2.7				
	2.85	17.7	0.18	4.3	40	1.5	+ 1.8	+ 0.9	E	9
3	1.68	15.7	0.17	4.4	188	7.9				
	1.61	16.2	0.18	4.0	240	9.0	+ 0.5	− 1.8	R	1
4	1.84	9.8	0.20	5.3	353	8.6				
	2.33	11.4	0.23	3.6	120	4.1	+ 1.6	− 0.6	H	1
	2.16	11.3	0.23	3.8	156	5.3	− 0.1	+ 0.2	R	1
	1.58	12.9	0.[illegible]6	4.3	[illegible]12	6.6	+ 1.6	+ 0.1	R	3
	2.11	11.5	0.24	3.2	64	1.7	− 1.4	− 1.2	E	10
5	2.13	15.7	0.17	4.3	166	9.4				
	1.75	16.4	0.18	4.3	300	14.2	+ 0.7	− 2.0	R	3
6	1.86	16.2	0.26	4.0	168	6.0				
	1.41	20.0	0.32	4.4	272	7.1	+ 3.8	− 0.1	R	1
	1.27	19.1	0.31	4.8	352	9.3	− 0.9	− 1.4	R	1
	1.38	20.0	0.33	3.7	231	6.1	+ 0.9	+ 1.6	H	6
	1.69	24.3	0.41	3.5	2[illegible]0	6.2	+ 4.3	+ 0.6	R	1
	1.82	24.3	0.41	2.9	[illegible]02	3.2	0	− 0.9	E	8
	1.99	20.2	0.35	3.6	92	2.6	− 4.1	− 1.6	E	5
	2.75	19.0	0.33	3.7	48	1.2	− 1.2	− 1.7	E	18
7	2.01	10.1	0.19	3.7	1[illegible]7	4.8				
	1.94	9.8	0.18	3.5	209	8.2	− 0.3	− 2.9	R	5
	1.88	10.5	0.19	3.6	48	1.3	+ 0.7	+ 2.0	E	10
8	1.27	14.2	0.17	4.0	370	1.9				
	1.47	13.9	0.17	4.9	226	1.7	− 0.3	+ 0.4	E	2
	1.52	16.1	0.20	3.9	100	1.5	+ 2.2	0	E	4
9	2.21	18.7	0.28	4.7	176	8.2				
	1.89	[illegible]0.5	0.31	4.3	238	8.8	+ 1.8	+ 0.4	R	1
	1.62	22.8	0.34	4.2	280	9.8	+ 2.3	+ 0.4	R	2
	1.74	17.3	0.27	4.1	120	2.9	− 3.5	− 3.2	E	8

R = retention phase E = excretory phase H = hemodialysis.

of duplicate determinations of K was 6.7 % and of VD_c 4.5 %

Glucose tolerance

The collected data from 33 intravenous glucose tolerance tests in nine patients with acute ischemic renal failure are presented in table II

It will be seen that the range of variations of the K values observed was 1.27—3.22 In only one instance was the K-value found to be above the lower limit

A well established form of temporary treatment of hyperpotassemia during anuria is the intravenous administration of hypertonic glucose with added insulin. The results of the present study would seem to indicate that the effect of this treatment may be expected to decrease with increasing azotemia, provided a constant ratio between net intracellular influx of potassium and glucose can be assumed.

Summary

In nine patients with acute ischemic renal failure glucose metabolism was studied by means of repeated intravenous glucose tolerance tests.

In twelve sequences during the retention phase, increasing blood urea concentration (BU) was associated with decreasing glucose tolerance in eleven instances.

In nine sequences during the excretory phase, decreasing BU was associated with increasing glucose tolerance in seven instances.

In three sequences, hemodialysis was followed by increased glucose tolerance in all instances.

Thus, in twenty-one of twenty-four sequences studied, increasing uremia was associated with decreasing glucose tolerance or vice versa.

The limitations inherent to the methods employed are stressed.

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determining true glucose, and hence is better suited for the accurate calculation of the slope of the glucose tolerance curve. In normal individuals the quantity of non glucose reducing substances is usually considered insignificant for most work on glucose tolerance but in uremic subjects, in which retention of non glucose reducing substances occurs these varying quantities of non-glucose reducing substances may well effect the slope of the glucose tolerance curve, if the total "glucose" concentration, as determined by the Hagedorn Norman Jensen method were used as the basis. This will not be the case if increment blood glucose values are used.

There are several inherent difficulties and shortcomings in studies of the present type. Thus the patients did not represent a homogenous group. Seven of the nine patients had undergone recent operations, three on the gastrointestinal tract two on the biliary tract and two on the urogenital system. One was the victim of a severe traffic accident and in only one instance was the development of acute anuria preceded by a relatively minor stress, an attack of biliary colic. It has been well established that temporary reduction of carbohydrate tolerance may follow all these types of stress. In addition infection, muscular unrest, low caloric intake and electrolyte disturbances are factors, which may effect the glucose assimilation rate in these patients. That such factors probably played a role in the present group of patients is evidenced by the fact that K was almost uniformly found lower than normal even when azotemia was not present (5).

However an attempt was made to minimize the effects of these factors. Thus, the tests were performed only when the patients were afebrile and at complete rest. Major electrolyte disturbances were not present as evidenced by the results of daily determinations of serum sodium, potassium, chloride and standard bicarbonate. The caloric intake was by necessity scanty during the retention phase, but usually adequate during the excretory phase when the patients were able to tolerate the diet well.

Therefore, since the effects of the factors mentioned would seem to be either minimal or to remain approximately constant during the period of the study it appears acceptable to attribute the observed fluctuations in glucose tolerance to the one factor which was similarly fluctuating the degree of azotemia.

The present work will therefore appear to confirm previous experience, that uremia is associated with an impairment of glucose tolerance (8 13) in the sense, that progressive uremia leads to increasing inhibition of glucose assimilation. But it furthermore provides evidence of the reversibility of this inhibition, since it was found in the majority of observations that reduction in the degree of azotemia whether this was effected through hemodialysis or through spontaneous diuresis, leads to improvement in glucose assimilation.

These changes in glucose assimilation, as measured by the intravenous glucose tolerance test, do not provide information on the mechanism of the underlying process, which, at the present can only be a matter of conjecture. It is worthy of note however that urea in concentrations comparable with those observed in this study may inhibit the action of certain enzyme systems concerned with protein metabolism (9). Whether enzymatic processes involved in glucose metabolism may be similarly influenced is not known.

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From the University Department of Pathology, General Hospital, Malmö, Sweden

Perforation of the Interventricular Septum in Myocardial Infarction

A Study Based upon an Autopsy Material

By

Sven Lundberg and Jan Söderström

Until a couple of decades ago perforation of the interventricular septum was still regarded as a rarity. In 1934 Sager (25) could only trace 18 cases in the literature, while in 1957 Gottsegen et al. (11) were able to collect as many as 172. In recent years further cases including several diagnosed ante mortem have been reported from Scandinavia (4, 12, 18, 19, 20, 21, 26, 30, 32) and other countries. Series of ruptures of the septum many of which were diagnosed before death have also been published (2, 13, 17, 24, 25, 31). All these series, however, probably represent certain selection. It is therefore questionable whether they warrant any conclusion as to the true frequency of the condition. Because of this it was decided to study a large necropsy series for the frequency of heart rupture, particularly perforation of the interventricular septum.

Material

Malmo is a town that lends itself well to epidemiological studies (3). The town is served by only one hospital, and in recent years almost 99 of all patients dying in hospital have been necropsied.

The files of the Department were studied for all cases in which autopsy had revealed signs of coronary sclerosis. In all cases with fresh gross or microscopic cardiac infarcts notes had been made on the extent of the lesion in the anterior wall, posterior wall or septum. In cases of heart rupture notes had been made on the site of the lesion and in cases with perforation of the interventricular septum the course of events before death was also studied in the hospital record cards.

The findings are summarized in table I which also includes cases of rupture of the papillary muscles, as these have importance in differential diagnosis. Table II shows how often the various parts of the wall of the left septum had been affected and table III, some clinical data and post-mortem findings in cases of perforation of the interventricular septum.

Discussion

It has gradually been realized that perforation of the interventricular septum is by no means so rare as was formerly supposed. But the true frequency of the condition cannot be deduced from the

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Table III Clinical data and necropsy findings

	Case no.					
	1	2	3	4	5	6
	Sex					
		♀	♀		♀	♂
	Age					
	76	88	76	81	74	75
Known hypertension before infarction	+	+	+	+	+	–
Probable age of infarction before perforation	Some days	Not known	5 days	Not known	Not known	Not known
Persistent hypertension after infarction	Yes 190/120		No	?	No	?
Systolic murmur noted	+	+	+	+	+	+
Probable survival after perforation	Some days	Some hours	2 days	3 days	At least 6 days	About 12 hrs
Septal perforation suspected	Yes	No	No	Later on	No	No
Anti-prothrombin treatment	Yes	No	Yes	Yes	No	Yes
Prothrombin index at perforation	104–78		50 – 26	92	–	82
Necropsy findings						
Extent of infarct	Post. + Sept. + Ant.	Post. + Sept.	Post. + Sept.	Ant. + Sept.	Ant. + Sept.	Post. + Sept.
Old scars		+				+
Heart weight (g)	570	400	430	600	360	490

currence of any further development of an infarct, but hardly decrease the risk of rupture of the septum.

Another point to be considered is that small perforations of the interventricular wall may be readily missed at autopsy unless special search be made for them (6, 23 24). In ruptures into the pericardium the presence of blood in the pericardium is sufficient to betray the occurrence of perforation. We consider this source of error less important because we always look for ruptures — also in the septum — in cases of recent infarction. Neither do we feel that the marked difference in the frequency of ruptures of the septum and the other two walls can be ascribed entirely to technical errors at autopsy

Heart rupture is said to be more common in the higher age classes (29) In the present material the average age of the patients who had died from rupture was also somewhat higher than that of those who had died from fresh infarcts without rupture. But the difference was negligible. The average age of those with rupture of the septum was still higher but the

Table I Survey of material 1957—1960

	Men	Women	Total
Autopsies (> 29 yrs)	1,811	1,845	3,656
Acute Infarctions	253 (14.0%)	183 (9.9%)	436 (11.9%)
Mean age (yrs)	68.3	74.6	71.0
Perforations other than of septum	23 (9.1%)	20 (10.9%)	43 (9.9)
Mean age (yrs)	70.0	75.7	72.7
Perforations of septum	1 (0.4%)	5 (2.7%)	6 (1.4%)
Mean age (yrs)	75.0	79.0	78.3
Rupture of papillary muscle	2 (0.8%)	2 (1.1%)	4 (0.9)
Mean age (yrs)	64.5	78.0	71.3

[1] Percentage of total no. of autopsies.
[2] Percentage of acute infarctions.

Table II Survey of acute infarctions (total 436)

	Anterior wall	Posterior wall	Inter ventricular septum
Site	247 (57%)	286 (66%)	236 (54%)
Ruptures	22 (8.9%)	21 (7.3%)	6 (2.5%)

number of cases on record because of an over representation of ante-mortem diag noses among the latter

Perforation of one of the walls of the heart was found in 11 % of all cases of fresh cardiac infarction This figure is compatible with those given by previous investigators viz 7 %—15 % (7 9 16 24 28 29 34)

Perforation of the septum was found to be much less common than rupture of any of the other walls in cardiac infarc tion (table II) This cannot be explained by the assumption that the surface area or the muscle mass of the septum is smaller than that of the anterior or posterior wall According to Roessle and Roulet (22) the muscle mass of the sep- tum is only slightly less than that of the anterior and posterior wall together It is true that the series of these authors consisted of normals below 50 years of age and that their findings are therefore not strictly applicable to our material in which cardiac hypertrophy was common and in which many of the patients were fairly old

We considered it more important to assess how often a wall with an infarct perforated We found that all three parts of the heart were equally often the site of an infarct and that the septum ruptured less frequently than the either of the other parts.

This difference may be attributed to various factors. Thus, the difference in pressure on either side of the muscle wall is believed to be an important factor (8, 17 25) This pressure gradient is, of course, much smaller between the left and right ventricle than that between the left ventricle and the pericardium. It has also been suggested that the numerous vascular anastomoses in the septum de- crease the risk of rupture there (25) In our opinion a dense network of vessels will probably counteract or prevent the oc

coming more and more aware of these so-called silent infarcts (29 33) In our cases 2 and 6 rupture was apparently not preceded by any symptoms, for they had not sought medical advice and they had not been admitted to hospital until after the perforation of the septum. In case 5 the patient had been in hospital for a fairly long time before death and neither infarction nor perforation of the septum had produced any demonstrable symptoms before death.

We are well aware of the difficulties in diagnosing rupture of the septum ante mortem and would only point out that the condition is more common than widely supposed. The clinical diagnosis might be made with the aid of the often characteristic findings. On analysis of the hospital records it was found that a loud systolic murmur had been noted in all of our cases, which, according to the literature, seems to be the most common and characteristic sign. Only in one case (No. 1) did the clinical records contain notes of suspected perforation of the septum before autopsy

Wider knowledge of the existence of the possibility of rupture of the septum in patients with an acute infarction is desirable because the duration of survival of such patients is sometimes fairly long According to the literature surgical correction of the defect has been attempted after rupture of the septum in at least 2 cases (6 27)

Summary

Analysis of the necropsies of 3 636 patients above 29 years revealed fresh infarcts in 436, including rupture into the pericardium in 43 and rupture of the septum in 6. The frequency of rupture of the interventricular septum was higher than could be deduced from published cases in which the diagnosis had often been made ante mortem. It may in the future be possible to diagnose these cases more frequently before death because they often develop a systolic murmur This symptom was noticed in the clinical records in all our cases.

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number of cases was not large enough to warrant any valid conclusions

Ruptures of the anterior and posterior wall respectively were equally common in both sexes, while rupture of the septum was much more common among women but the group was small (6 cases) and no such dominance for women has been found in the literature However the figures in the literature may have been based on primary series with an over representation of males.

Hypertension is believed to increase the risk of perforation (8, 10 24 29) In most of our cases the blood pressure had been increased before the fatal infarct Only in one patient (No 6) had the blood pressure recordings noted before the final attack been repeatedly normal. An increased blood pressure even after infarction is said to favour rupture (8, 10 29) We were able to demonstrate this in only one of our cases (No 1)

Overweight of the heart and hypertrophy of the left half of the heart were noted in all cases except one (No 5) in which the weight of the heart was only slightly above normal The kidneys invariably showed some degree of nephrosclerosis suggesting that the patients had had hypertension. In case No 1 however the kidneys could not be examined histologically but the gross appearance of the organs was compatible with nephrosclerosis It is, however difficult to ascertain the frequency of hypertension in these cases for mild hypertension is often seen in association with arteriosclerotic changes, which can in turn cause infarcts, which may rupture.

It is widely believed that treatment with anticoagulants increases the risk of rupture (1 14 16 28) Anticoagulants had been used in only 4 of our cases and only in one (No 3) had treatment resulted in a reduction of the prothrombin index before the assumed time of the rupture.

It is not always easy to reconstruct the true course of a fatal disease from the notes in a patient's record sheets. We shall therefore confine ourselves to a brief comment on some clinical findings. Nor is it intendent to discuss the criteria of septum rupture and the differential diagnosis. The reader is instead referred to special papers on the subject (2, 5 13, 17 20 24 25 29 31) As mentioned previously an ever increasing number of cases of perforation of the septum has been diagnosed ante mortem. It has been claimed that if infarction is followed by a marked rapid general deterioration, possibly with increased pain and pronounced right sided cardiac failure, septum rupture may be suspected. On auscultation a loud systolic murmur is heard, maximal over the left lower border of the sternum despite the patient's poor general condition and the low blood pressure. As early as 1846 Latham (15) who described the first case of perforation of a septal infarct, mentioned the clinical findings, but not until 1923 was it possible to diagnose the condition ante mortem (5)

It is of particular interest that patients often survive rupture of the septum longer than perforation into the pericardium (1 8 24) Survivals of 4—5 years have been reported by Wood and Livezey (35) and Zucker et al (36) The diagnosis has sometimes been made by catheterization (6 11 19 27) None of our patients survived so long (table III)

Though it is not intended to discuss the clinical symptoms of perforation of the septum here, it should be stressed that the infarct responsible for rupture need not produce any clear or classical symptoms. Both clinicians and pathologists are be-

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Nephrotoxic Effect of Phenacetin and Acetylsalicylic Acid in Animal Experiments

By

Erna Clausen

Whether analgesics possess a nephrotoxic effect has been a matter of lively discussion since Spühler and Zollinger (8) in 1953 pointed out that a causal relationship might exist between phenacetin medication and chronic interstitial nephritis. Clinical findings indicate a correlation between the intake of analgesics and renal damage.

The animal experiments reported so far (1 2, 4 5 6 9 10, 11) have not afforded an unambiguous answer to this question. Miescher et al. (5) succeeded in inducing interstitial nephritis in rabbits by combining phenacetin administration with intravenous injection of E. coli, whereas injection of E. coli alone did not affect the kidneys. Similar experiments were later performed by Miescher et al. (6) Keller et al. (4) and Bartels et al. (2) but with negative results.

Thus, the question regarding a damaging effect of analgesics upon the kidneys still remains unelucidated and the problem is of such topical interest that it calls for further investigations.

Phenacetin has so far been considered the harmful agent. Short-term provocation experiments on man suggest that acetylsalicylic acid is equally nephrotoxic (3).

The present study was designed to reproduce Miescher's (5) original result and, on the basis of the above-mentioned experience of acetylsalicylic acid to ascertain by animal experiments whether this agent combined with coli infection is able to produce histologically demonstrable changes in the kidneys.

Material and method

The experimental animals were rabbits, which are supposed to possess the advantage over mice and rats of seldom acquiring spontaneous urinary infections. Ten rabbits were fed phenacetin, 10 acetylsalicylic acid, and 8 placebo (fig. 1). A placebo group was preferred to an ordinary control group, since the stress of the feeding might be imagined to influence the rabbits' growth.

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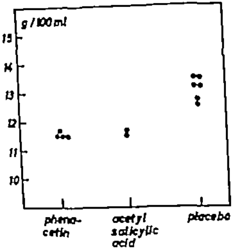


Fig. 3. Haemoglobin level (g/100 ml) after approx. 11 months' feeding (before inoculation with E. coli).



Fig. 4. Serum creatinine concentration (mg/100 ml) after approx. 11 months' feeding (before inoculation with E. coli).

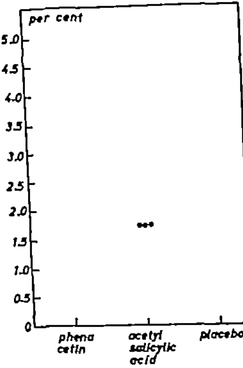


Fig. 5. Methaemoglobin level after approx. 11 months' feeding (before inoculation with E. coli).

The weight gain in the phenacetin and acetylsalicylic acid groups differs significantly from that in the placebo group ($P < 0.001$ %)[1]

Two rabbits (Nos. 15 and 19) died two months before the experiment was concluded and have to be left out of the analysis.

Fig 3 shows the haemoglobin concentrations in the 3 groups. The levels were lower in the phenacetin and acetylsalicylic acid groups than in the placebo group (lowest in the phenacetin group) but with a marked scatter in the individual groups. The difference is significant ($P < 0.001$ %)

Correspondingly the serum creatinine concentration (fig 4) was significantly higher in the phenacetin and acetylsalicylic acid groups than in the placebo group ($P = 2$ ‰)

After the coli infection there was in most cases an increase in the serum creatinine concentrations from 0.1 to 1 43 mg/100 ml.

[1] The statistical calculations were carried out by N. F Gjeddebæk, Actuary Ferrosan Ltd., Copenhagen.

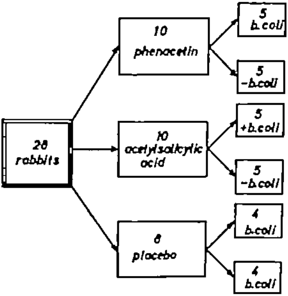


Fig 1 Survey of the experimental groups.

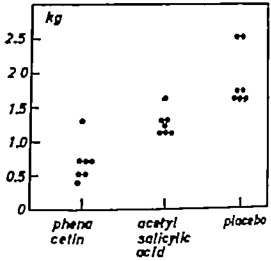


Fig 2. Weight gain (kg) during the period of medication. Rabbits, initial weight approx. 2.5 kg.

In previous experiments of this kind the drugs have been mixed with the feed or administered by gastric tube. In the present experiments they were administered in the form of tablets placed by forceps on the posterior part of the tongue, thus eliciting a swallowing reflex. This method is more accurate than mixing the drugs in the feed and gentler than tube feeding

The phenacetin and acetylsalicylic acid tablets were made from raw materials as prescribed in the Danish Pharmacopoeia, the phenacetin tablets having a 4-chloracetanilide content of less than 0.35 % The placebo tablets were made of lactose and potato starch.

Tablets were administered on all weekdays. The initial dose was 1 g daily of phenacetin, acetylsalicylic acid, and placebo respectively increasing to 1.5 g daily Each rabbit received a total of 325 g of one of the named substances. At the end of almost 11 months half the rabbits of each group (fig 1) were inoculated with E. coli[1] a total of 10^8 distributed between two intravenous injections with an interval of two days. Six days after the first injection all the rabbits were killed.

Before the injection of E. coli determinations of haemoglobin, serum creatinine, and methaemoglobin were carried out on most of the rabbits. The serum creatinine concentration was again determined in the infected rabbits before they were killed.

The animals were autopsied, the kidneys measured, cut, and fixed in formalin. The specimens were stained, partly with haematoxylin-eosin and partly by the method of van Gieson-Hansen.

In order to make the histological examination of the kidneys as objective as possible, the preparations were examined by two investigators and, after having been examined in known sequence, mixed at random and examined again in unknown sequence. The degree of infiltration with polymorphonuclear leukocytes and round cells was graded from 0 to ++++

Results

After 9—10 months feeding the rabbits in the phenacetin and acetylsalicylic acid groups began to look ill, had a poor appetite, and lost weight. All had weighed about 2.5 kg when the experiment was started. Before they were sacrificed the rabbits in the two first groups ranged from 3 to 4 kg and the placebo rabbits from 4 to 5 kg Fig 2 gives the weight gain in the 3 groups.

[1] The coli strain was O 656/60, known to be toxic. It was kindly supplied by Dr Fritz Ørskov Statens Seruminstitut, Copenhagen.

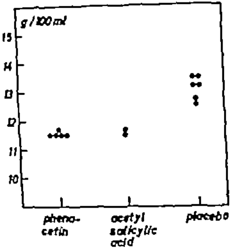


Fig. 3. Haemoglobin level (g/100 ml) after approx. 11 months' feeding (before inoculation with E. coli).

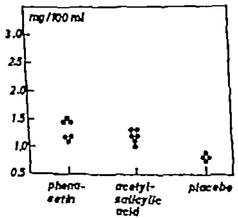


Fig. 4. Serum creatinine concentration (mg/100 ml) after approx. 11 months' feeding (before inoculation with E. coli)

The weight gain in the phenacetin and acetylsalicylic acid groups differs significantly from that in the placebo group (P < 0.001 %)[1]

Two rabbits (Nos. 15 and 19) died two months before the experiment was

[1] The statistical calculations were carried out by N. F Gjeldebæk, Actuary Ferrosan Ltd., Copenhagen.

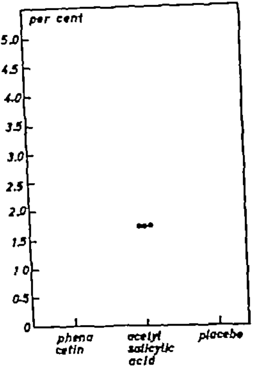


Fig. 5. Methaemoglobin level after approx. 11 months' feeding (before inoculation with E. coli).

concluded and have to be left out of the analysis.

Fig 3 shows the haemoglobin concentrations in the 3 groups. The levels were lower in the phenacetin and acetylsalicylic acid groups than in the placebo group (lowest in the phenacetin group) but with a marked scatter in the individual groups. The difference is significant (P < 0.001 %)

Correspondingly the serum creatinine concentration (fig 4) was significantly higher in the phenacetin and acetylsalicylic acid groups than in the placebo group (P = 2 %)

After the coli infection there was in most cases an increase in the serum creatinine concentrations from 0.1 to 1.43 mg/100 ml.

Table I Gross and histological findings in the kidneys of 28 rabbits

No.	Drug	Inoculation with E. coli	Gross inspection. Measurements (cm)			Histological changes
			Right	Left		
1	Phenacetin	+	4×3×2	4×2¹/ ×2		++
2	Phenacetin	+	4×3×2	4×3×2	Yellowish infiltrations in the papilla	+++
4	Phenacetin	+	4×2 / ×1 /	4×2 / ×1 /	Several abscesses on surface and cut surface	++++
5	Phenacetin	+	4×3×2	4×3×.	Several abscesses on surface and cut surface	++++
6	Phenacetin	+	4×3×2	4×3×2		++++
3	Phenacetin	—	4×3×2	4×3×2		+
7	Phenacetin	—	4×2 / ×2	4×2¼ ×2		+
8	Phenacetin	—	3 / ×2 / ×1 /	3¹/ ×2 / ×1 /		+
9	Phenacetin	—	4×3×2	4×3×2		+
10	Phenacetin	—	3 / ×2 / ×2	3¹/ ×2 /ₐ×2		(+)
14	Acetylsalicylic acid	+	4×2 / ×2	4×2¹/ₐ×2	A few small abscesses on the surface and cut surface	++++
16	Acetylsalicylic acid	+	4×2 / ×2	4×2¹/ ×2		+
17	Acetylsalicylic acid	+	4×2 / ×2	4×2¹/ ×2		++
18	Acetylsalicylic acid	+	4×2 / ×2	4×2¹/ ×2		+++
20	Acetylsalicylic acid	+	4×3×2	4×3×2	A few streaked infiltr in the papilla	++
11	Acetylsalicylic acid	—	4×3×2	4×3×2		+
12	Acetylsalicylic acid	—	4×3×2	4×3×2		+
13	Acetylsalicylic acid	—	4×3×2	4×3×2	Scars on the surface	+++
15	Acetylsalicylic acid	—				—
19	Acetylsalicylic acid	—				—
21	Placebo	+	4×2 / ×1 /	3¹/ ×2×1 /		+
22	Placebo	+	4×2 / ×1¼	4×2¹/ ×1¼		—
25	Placebo	+	4×2¼ ×1 /	4×3×2		—
26	Placebo	+	4×2 / ×2	4×3×2		—
23	Placebo	—	4×2¹/ ×1 /	4×2 / ×1 /		—
24	Placebo	—	4×2 / ×1¼	4×2 / ×1 /		—
27	Placebo	—	4×2¹/ ×1 /	4×2 / ×1 /		+
28	Placebo	—	3 / ×2×1 /	3 / ×2×1 /		+

— = no abnormality (+) = negligible, + = mild, ++ = moderate, +++ = severe, interstitial and perivascular infiltration with polymorphonuclear leukocytes and round cells. ++++ = very severe interstitial and perivascular infiltration with polymorphonuclear leukocytes round cells and abscesses.

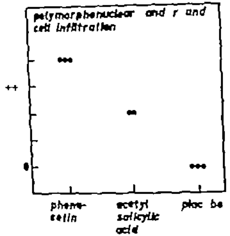


Fig. 6. Microscopic findings after inoculation with E. coli. The pluses indicate the degree of infiltration with polymorphonuclear leukocytes and round cells (cf. table I).



Fig. 7. Rabbit No. 4 (phenacetin + coli) 4 × 2 1/2 × 1 1/2 cm. Abscesses on the surface of the kidneys.

Rabbits are generally not assumed to form methaemoglobin (7) but the present study indicates that they do. The values showed a marked dispersion in the individual groups. Although there was not a significant difference between the values in the phenacetin and acetylsalicylic acid groups as compared with the placebo group, there was a tendency to higher values in the former two (fig. 5).

Pathology

Table I sets out the chief gross and histological findings. The kidneys of all groups were of equal size. In the phenacetin-coli and in the acetylsalicylic acid-coli groups there were in some cases gross abscesses on the surface and the cut surface of the kidneys (fig. 7).

Histological examination of the kidneys in the phenacetin-coli and acetylsalicylic acid-coli groups showed predominantly acute changes in the form of interstitial and perivascular infiltration with polymorphonuclear leukocytes and round cells, increasing to abscess formation, interstitial hyperaemia and oedema, in places dilatation of the convolutions and collecting tubules, and in a few sites a mild increase in connective tissue. In a few cases bacteria were seen in the preparations.

The non-inoculated phenacetin and acetylsalicylic acid groups showed similar but less pronounced histological changes of the kidneys (but never bacteria).

In the mild cases the infiltration was predominantly localized to the cortico-medullary junction, while in the more severe cases it was scattered throughout both cortex and medulla.

In the placebo-coli group and in the non-inoculated placebo group there was mild scattered infiltration with round cells and polymorphonuclear leukocytes in 1 out of 4 and in 2 out of 4 rabbits respectively.

There was a significant difference between the infiltration with polymorphonuclear leukocytes and round cells in the phenacetin-coli and acetylsalicylic acid-coli groups on the one hand and the placebo-coli group on the other ($P < 0.001$ /₄) (fig. 6), while there was no significant difference between the polymorphonu-

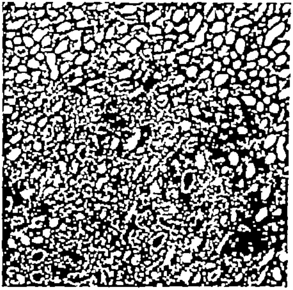


Fig 8. Rabbit No. 4 (phenacetin + coli) Very severe interstitial infiltration with polymorphonuclear leukocytes and round cells in the medulla. Dilatation of collecting tubules.



Fig 10 Rabbit No. 14 (acetylsalicylic acid + coli) Severe perivascular infiltration with polymorphonuclear leukocytes and round cells at the cortico-medullary junction. Dilatation of convolutions and collecting tubules.



Fig 9 Rabbit No. 8 (phenacetin, but not coli) In places infiltration with polymorphonuclear leukocytes and round cells at the cortico-medullary junction.

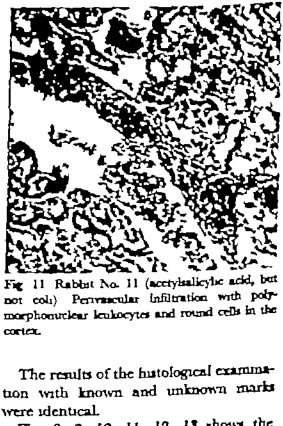


Fig 11 Rabbit No. 11 (acetylsalicylic acid, but not coli) Perivascular infiltration with polymorphonuclear leukocytes and round cells in the cortex.

clear and round-cell infiltration in the non inoculated phenacetin and acetylsalicylic acid groups and the non-inoculated placebo group

The results of the histological examination with known and unknown marks were identical.

Fig 8, 9 10 11 12, 13 shows the histological renal changes in the various groups.

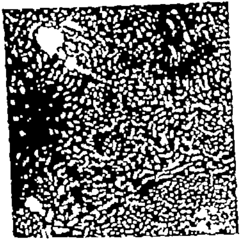


Fig. 12. Rabbit No. 21 (placebo + coli). In few sites negligible interstitial infiltration at the cortico-medullary junction.

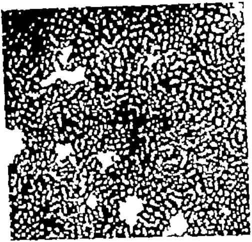


Fig. 13. Rabbit No. 27 (placebo, but not coli). In one place negligible interstitial infiltration in the cortex.

Discussion

This experiment revealed that rabbits pre-treated with large doses of phenacetin or acetylsalicylic acid and then inoculated with E. coli developed inflammatory changes in the kidneys which did not occur in coli-inoculated rabbits on placebo medication.

Although the moderate histological changes in the non-inoculated phenacetin- and acetylsalicylic acid groups did not differ significantly from those in the non-inoculated placebo group there was a difference. In the first two groups there was hyperaemia, oedema, dilatation of the convolutions and collecting tubules and in some cases increased connective tissue, while in the third group there was only negligible infiltration with polymorphonuclear leukocytes and round cells in otherwise normal renal tissue.

The result of this experiment appears to confirm the clinical assumption that phenacetin damages the kidneys, and it also confirms Miescher's (5) original findings. It is interesting that acetylsali-cylic acid (3) apparently exerts a similar effect, though less marked. The weight gain was somewhat greater, the haemoglobin levels a little higher and the serum creatinine concentrations a little lower in the acetylsalicylic acid group than in the phenacetin group. Moreover the histological changes were a little more severe in the phenacetin-coli group than in the acetylsalicylic acid-coli group.

This leads to the question of how these substances damage the kidneys, if they do possess a specific nephrotoxic effect.

The poorer general condition, the less marked weight gain, the lower haemoglobin levels, and the higher serum creatinine concentrations in the phenacetin and acetylsalicylic acid groups may be due either to a direct nephrotoxic effect of the respective drugs, as indicated particularly by the elevated serum creatinine, or to a separate effect of the two drugs, when administered in sufficiently great doses, reducing the resistance to

bly the animals have occasional bacteriaemia or are exposed to ascending urinary infections. If the organism is weakened or if the kidneys in particular are damaged, such incidents may give rise to mild histological inflammatory changes as found in the non inoculated phenacetin and acetylsalicylic acid groups of the present experiment.

Bacteriological autopsies were not performed, but the gross inspection showed no signs of sepsis. Hepatic and pulmonary tissue was removed in all cases. These structures showed histological signs of mild infiltration with polymorphonuclear leukocytes and round cells in some cases, but not particularly in the groups inoculated with E. coli. In phenacetin and acetylsalicylic acid treated rabbits the kidneys are evidently particularly sensitive to infection, thus constituting a *locus minoris resistentiae*

Summary

The object of the present experiments was to investigate whether phenacetin and acetylsalicylic acid combined with coli infection gives rise to histological renal changes.

Twenty-eight rabbits, divided into 3 groups (fig 1) received phenacetin, acetylsalicylic acid and placebo respectively for approx. 11 months. Thereupon, half the rabbits of each group were infected with a known coli strain by intravenous injection.

In the phenacetin-coli and acetylsalicylic acid-coli groups histological examination of the kidneys revealed acute changes consisting in interstitial and perivascular infiltration with polymorphonuclear leukocytes and round cells increasing to abscess formation interstitial hyperaemia and oedema, dilatation of the convolutions and collecting tubules, and in places slightly increased connective tissue. The non-inoculated phenacetin and acetylsalicylic acid groups showed similar but less marked histological changes in the kidneys. In the placebo-coli group and in the non-inoculated placebo group there was mild, scattered infiltration with round cells and polymorphonuclear leukocytes in 1 out of 4 and in 2 out of 4 rabbits respectively

A significant difference was found between the weight gain, haemoglobin levels, and serum creatinine concentrations in the phenacetin and acetylsalicylic acid groups on the one hand and the placebo group on the other

It is discussed whether the renal damage is due to a direct nephrotoxic effect of the two drugs or whether the drugs affect the rabbits general condition, reducing their resistance to infections, especially renal infections.

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Metabolic Effects and Diagnostic Value of Small Doses of Folic Acid and B_{12} in Megaloblastic Anemias[1]

By

Harald A. Hansen and Alexander Weinfeld

It is known that there exists an interaction between vitamin B_{12} and folic acid and that a therapeutic trial with generally accepted doses of these substances cannot always distinguish between megaloblastic anemia due to B_{12} and that due to folic acid-deficiency (1 2) However it is clinically and biochemically evident that these two vitamins are specifically different in action (3 4 5 6, 7) The therapeutic non-specificity of pteroylglutamic acid (PGA) in megaloblastic anemias led Vilter et al. (8) to the hypothesis that the hematological cross-responses could be due to a "mass action" Marshall and Jandl 1960 (9) drew the consequences of this hypothesis and studied the reticulocyte response during treatment with 0.4 mg PGA a day parenterally In 3 cases of pernicious anemia and 3 cases of folic acid-deficiency anemia they found a different reticulocyte response. These authors recommended the use of 0.2—0.5 mg PGA i. m. a day as a diagnostic therapeutic trial in suspected folic acid-deficiency anemia. The daily requirements of folic acid are still not known and the doses mentioned were chosen by the authors on the basis of folic acid excretion studies (10) These doses were also regarded as sufficient for the prevention of dietary deficiency of folic acid without endangering patients with undiagnosed pernicious anemia.

The present investigation was designed to study the effects of small doses of folic acid and B_{12} in megaloblastic anemias, with regard to the metabolic changes as well as to blood morphological responses with special reference to the differential diagnostic value. How much folic acid can be given during a long time to a patient with intrinsic factor deficiency without masking his inherent pernicious anemia and hence risking neurological complications is also discussed (11 12, 13 14 15)

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Methods

Reticulocytes were stained with brilliant cresyl blue and counted in a dry preparation as described by Seip (16) The absolute number of reticulocytes was calculated from a simultaneous red cell count. Bone marrow examinations were done on sternal aspirates and the erythropoiesis was classified as totally partially and intermediate megaloblastic. Giant myeloid cells were counted as previously described (17) The methods for estimation of hemosiderin and sideroblasts in bone marrow determination of serum iron and iron-binding capacity (TIBC) were as stated elsewhere (18) Haptoglobin in serum was estimated as the total hemoglobin-binding capacity (THbBC) according to Jayle as described by Nyman (19) Bilirubin in serum was determined by a slight modification of the Jendrazik method (20)

The gastric juice was usually examined after a histamine test dose of 0.5 mg A maximal histamine stimulation was done in some cases.

Vitamin B_{12} in serum was assayed with Euglena gracilis strain Z according to Hutner et al. (21) The vitamin B_{12} resorption tests were done according to Schilling using a carrier dose of 1 0 μg vitamin B_{12} (22) 24-hour as well as 48-hour urine samples were examined for radioactivity In cases with subnormal excretion of labeled vitamin B_{12} the test was repeated with simultaneous administration of intrinsic factor

Folic acid derivatives in *whole blood* were assayed with Lactobacillus casei and Streptococcus faecalis after addition of ascorbic acid as previously described (23) A slight modification was however adopted in these series as the protein precipitation was carried out in an autoclave at 119° for 10 min. using a blood dilution 1/10 (3 ml whole blood was added to 21 ml distilled water After hemolysis 6 ml of 0.25 M phosphate buffer solution pH 6.1 containing 300 mg / 1 ascorbic acid was added) Blood specimens were drawn in the morning and the hemoglobin concentration was adjusted to about 12 ± 1 g % by removing plasma if anemia was present. Heparin blood (1 mg heparin sulphate per test tube as a dry film) was often used in this study because it is more precise than the technic with citrated blood when concentration procedures are necessary Folic acid in serum was assayed with L. casei with the same technic as for whole blood, but using the phosphate-ascorbic acid solution for serum dilutions as described by Baker et al. 1959 (24) If the filtrates were opalescent a protein precipitation was done at pH 5 0 which does not entail any loss of folic acid activity The final dilutions used were 1.80, 1.200 1·400. Usually the samples were analysed on two different occasions. The median value and the normal range for whole blood and serum are given in table I.

Formiminoglutamic acid (FIGlu) excretion in the urine after a 1 histidine load was measured according to Tabor and Wyngarden (25). In most cases the patients were given 5 g 1-histidine 3 times daily during 3 consecutive days and their 24-hour acidified urine samples were examined. In the later part of this investigation the FIGlu excretion was measured after a single fasting morning dose of 15 g l histidine. No food was allowed during the next two hours. The first hour urine portion was discarded and the urine was subsequently collected during 8 hours for FIGlu examination. The acidified urine was stored at + 4 or if longer preservation was necessary at — 20 The amount of FIGlu excreted was calculated from the molar extinction coefficient given by Tabor and Wyngarden (25). This figure was later checked by a reference sample of Ba FIGlu kindly supplied by Dr Tabor and good agreement was obtained. Healthy controls excrete less than 10 μM/hr on the 3-dose schedule and less than 28 μM/hr after the single histidine dose.

Characterization of the material and principles of treatment

Seventeen patients with Addisonian anemia and 9 patients with megaloblastic anemia due to folic acid deficiency were studied. The pertinent diagnostic values for all patients are presented in table I As is seen from the table a clear-cut separation of the group of pernicious anemia from th group of megaloblastic anemia due to folic acid deficiency can unequivocally be done by the laboratory data obtained. Some supplementary comments are given below

All cases but one (L. R.) with *pernicious anemia* had normal folic acid levels in serum.

The folic acid content in *whole blood* assayed by L. casei was subnormal in 6 cases and in 2 other cases (A. K. and A. J.) was definitely within the folic acid deficiency range (< 15 mμg/ml). The S. faecalis activity was low in 2 cases. FIGlu excretion was increased in 9 cases. In 3 cases the FIGlu excretion was extremely high. Other laboratory tests applied in these patients satisfied the diagnosis of uncomplicated Addisonian pernicious anemia.

The cause of *folic acid deficiency* was in 3 cases general malabsorption, and in one case malnutrition combined with alcoholism, in 5 cases the deficiency was of unknown etiology. Two of the last mentioned cases have been treated for many years with phenobarbitone or diphenylhydantoin preparations for epileptic seizures. A third was treated with sulphonamides for more than year because of chronic infection of the urinary tract. Four of the patients with folic acid deficiency anemia had subnormal B_{12} levels in serum. Three of the last mentioned had general malabsorption syndrome and showed no increase in the B_{12} absorption of labeled B_{12} on addition of intrinsic factor. Two cases had extremely low serum folic acid levels but their whole-blood folic acid content was only somewhat subnormal, probably due to blood transfusion before assay. All patients in group II had free HCl in the gastric juice.

Before therapy was instituted serial observations of the reticulocyte number were made. The control period was usually 3 days. Longer observation was not considered necessary as the reticulocyte values during the first days of treatment could also be regarded as basic values. All patients were initially treated with small doses of either PGA (Folvite® Lederle) or vitamin B_{12} as detailed below. If no reticulocyte response was obtained after the initial treatment the therapy was changed to the other vitamin in the doses outlined below. Before changing therapy examination of blood chemistry and sternal marrow was usually repeated.

Group I. Pernicious anemia

The 17 patients in this group were subdivided into

Group I 9 patients who were initially treated with daily i. m. injections of small doses of PGA (0.1—0.4 mg) and subsequently with small doses of B_{12} (2—5 μg)

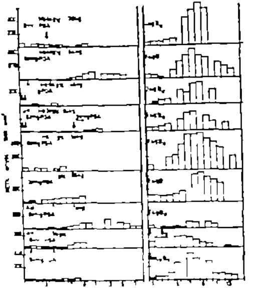


Fig. 1. The reticulocyte response in pernicious anemia initially treated with small doses of PGA and subsequently treated with small doses of B_{12}. Arrows indicate transfusions of packed red cells.

Group I b 5 patients were initially given small doses of B_{12} (3 μg) daily followed by large doses of B_{12} (100—1000 μg)

Group I c: 3 patients were treated orally with multivitamin preparation containing PGA corresponding to 1—3 mg PGA day

Group II. Folic acid deficiency

Of the 9 patients with *folic acid-dependent megaloblastic anemia* 4 were treated first with either small or large doses of B_{12} and then by small doses of PGA. In the other 5 cases treatment was started with small doses of PGA. In all 9 patients treatment with small so-called "physiologic" doses of PGA (0.1—0.4 mg) was followed by therapeutic so-called "pharmacologic" doses, i. e. 15 mg PGA i. m. a day

Results

Reticulocyte count

The reticulocyte response in group I a) to small doses of PGA (1.5—7 μg/kg body weight) is seen from fig. 1. Of the 9

Table I Diagnostic values for all patients studied

Subject Sex	Age	Hb g % 14.1 (12.1–16.1)	RBC mill/mm³ 4.6 (4.0–5.2)	Serum iron µg % 129 (50–240)	TIBC µg % 310 (250–400)	Bili-rubin mg % ≤ 1.1	THbBC mg % 110 (30–190)	Serum B_{12} pg/ml 340 (200–625)	Serum folic acid mµg/ml 5.4 (3.7–9.5)
Group I a. Pernicious anemia initially treated with physiologic doses of PGA									
A. B., ♂	47	7.6	2.0	230	265	1.5	4	50	3.9
V. A., ♀	54	5.5	1.5	145	185	0.9	2	45	6.3
F. O., ♂	71	6.1	1.9	215	350	1.2	0	30	5.2
Hj. J. ♂	61	4.7	1.2	235	245	1.4	0	10	6.0
L. R., ♀	85	6.6	2.0	190	255	0.8	0	30	14
G. K., ♂	48	3.9	1.2	260	300	1.3	0	20	4.0
A. K., ♀	72	5.6	1.2	240	260	1.6	0	45	—
L. F., ♀	54	5.1	1.1	250	265	1.0	0	20	—
E. A., ♀	38	4.6	1.3	210	290	1.9	2	35	7.3
Group I b. Pernicious anemia initially treated with small doses of B_{12}									
L. B., ♀	71	9.4	2.2	175	280	1.3	23	80	3.1
H. H., ♀	72	9.5	2.4	170	275	0.8	4	25	8.2
E. J., ♀	67	8.9	2.4	125	255	0.9	166	100	3.2
M. M., ♀	61	10.6	2.9	90	300	—	30	40	3.7
Ch. M., ♀	69	5.6	1.6	200	220	0.9	0	20	3.9
Group I c. Pernicious anemia treated with 1–3 mg PGA orally a day									
H. S., ♀	79	9.1	3.1	235	290	—	0	40	9.1
A. J., ♀	77	6.0	1.6	205	265	1.2	0	90	3.9
A. K., ♂	57	4.4	1.5	230	330	1.5	4	15	5.9
Group II. Megaloblastic anemia due to folic acid deficiency									
K. H., ♀	63	4.2	1.0	270	363	0.7	0	150	0.5
S. P., ♀	73	5.8	1.2	190	310	1.0	0	175	0.5
M. L., ♀	26	5.4	0.9	210	240	0.3	0	235	0.7
L. R., ♀	26	10.2	3.4	190	250	—	0	110	0.7
I. J., ♀	40	6.4	2.2	130	335	1.0	0	120	1.5
A. S., ♀	76	5.4	1.3	170	205	1.3	0	20	1.2
Kv J ♂	78	6.5	1.7	90	—	—	–	100	0.4
		7.4	1.9	—	—	—	6	340	0.4
G. F., ♀	56	7.9	1.9	185	220	0.6	0	240	1.2
G. A., ♂	76	7.4	2.1	230	230	0.6	0	540	1.9

¹ Blood transfusion was given some days before the examination was done.
FIGlu excretion examined after a single load of 15 g l-histidine. Normal values 11.8 (5.4–27).

...hole blood ...lic acid		FIGlu µM/hr < 10	Free HCl	Schilling test > 8 %	Schilling's test with intr. factor	Bone marrow			
... ...ed m/µg/ ...l 47 (23– ...)	S. folic m/µg/ ml 2.8 (1.0– 10)					Erythropoiesis*	Giant stabs /∞	Sideroblasts % 42 (12–80)	Hemosiderin 0–+++
13	0.9	20	0	4.0	21.0	P M.	70	75	++
21	3.4	16	0	3.7	25	M.	80	80	+
22	3.7	—	0	4.8	25	M.	70	82	++
17	1.5	127	0	1.8	11	M.	80	78	++
36	0.5	63	0	1.4	20	M.	260	84	+++
26	2.0	245	0	4.4	24	P M.	160	88	++
12	1.6	9	0	1.5	35	P M.	120	57	++
24	1.7	15	0	3.6	28	M.	180	85	+
46	5.6	58	0	0	—	M.	270	72	++
21	1.1	4	0	0.6	19	P M.	110	84	++
30	0.8	—	0	2.7	21	P M.	20	57	+
16	1.1	4	0	5.7	31	P M.	35	72	++
24	2.9	—	0	5.6	—	L M.	15	92	++
15	—	25	0	4.5	—	M.	90	60	++
23	1.0	—	0	—	—	P M.	33	84	++
13	0.2	—	0	1.9	12.4	M.	60	90	+
27	6.9	30	0	2.5	27	M.	100	90	+++
3.3	0.8	45	+	27	—	M.	140	94	++
6.6	0.9	18	+	23	—	P M.	50	80	++
19	0.8	103	+	—	—	M.	170	80	++
7.1	0.4	—	+	—	—	Makrobl.	5	0	0
17	2.4	*840	+	0.8	1.8	P M.	40	90	+++
5.7	0.4	*67	+	9.1	6.6	M.	170	74	++
11.0	0.9	—	(+)			M.	40	92	+++
12.4	5.7	340		7.8	14.6	L M.	<5	77	+++
12.0	1.3	*39	+	17	—	P M.	60	90	++
12.0	0.4	76	+	30	—	P M.	60	100	+++

M = megaloblastic. P.M. = partially megaloblastic. I.M. = intermediate megaloblastic.
Sternal puncture was performed 3 months earlier at which time the patient had spontaneous remission. Later on relapse occurred and the diagnosis of non-tropical sprue became feasible.

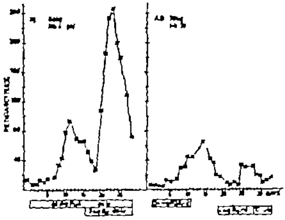


Fig 2 The reticulocyte response to small doses of PGA and subsequently small doses of B_{12} in two cases of uncomplicated pernicious anemia.

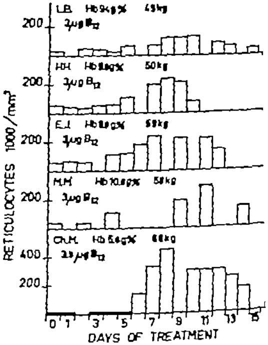


Fig 3. The reticulocyte response in pernicious anemia initially treated with small doses of B_{12}. No secondary reticulocyte peak was obtained in subsequent therapy with large doses of B_{12}.

patients examined 5 had an unchanged reticulocyte count However in 4 cases the reticulocytes increased significantly within 8 to 10 days of treatment although their reticulocyte peak was lower than expected from the hemoglobin and red cell values. All patients responded with an expected reticulocyte peak when PGA treatment was changed to small doses of B_{12} (2—5 μg/day) Even in those cases who showed some reaction during PGA treatment the most striking feature was the secondary greater reticulocyte response during subsequent B_{12} treatment. Only in one case (A. B.) was the secondary reticulocyte response meager In this patient who was given 0 4 mg PGA during 10 days the hemoglobin concentration increased and the haptoglobin value became normal before B_{12} therapy was instituted on the 19th day That a diagnostic trial limited to PGA treatment with doses of 0 4—0 2 mg/day may give an erroneous impression as to the etiology of the megaloblastic anemia may be seen from fig 2

The 5 patients in group I b) who were treated initially with small doses B_{12}, all responded to 3 μg B_{12} a day (fig 3) The reticulocyte response was somewhat lower than in the former group because of the higher hemoglobin level in these patients. No secondary reticulocyte peak was noted during subsequent therapy with large doses of B_{12}

Fig 4 shows the reticulocyte response to oral doses of PGA in group I c. The reticulocyte response was similar to that expected in pernicious anemia treated with pharmacologic doses of vitamin B_{12}. These patients showed also the expected rise of the red cell count after 15 days of therapy (26)

In folic acid deficiency (group II) 8 patients responded optimally to small doses of PGA (fig 5) Their reticulocyte peak was comparable to that obtained in patients with pernicious anemia treated with small doses of B_{12}. One patient (G A.) did not respond to 0 1 mg PGA (1.2 μg PGA/kg body weight) but reacted

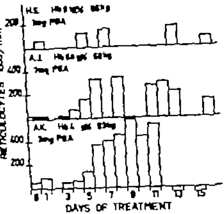


Fig. 4. The reticulocyte response in pernicious anemia treated with 1—3 mg PGA day by mouth. No secondary reticulocyte peak was seen after subsequent therapy with large doses of B_{12} parenterally

with suboptimal reticulocyte response to 0.2 mg PGA a day. His erythropoesis was normoblastic after 10 days of therapy. However the hemoglobin concentration did not rise and it became evident during the further observation of the patient that the treatment failure was due to complicating disease.

Serum iron and iron-binding capacity

In six of the patients with pernicious anemia (group I a) the serum iron concentration fell during treatment with small doses of PGA (fig. 6). Four of these patients had concomitantly shown a slight rise in the reticulocyte count. Patient G. K. who was treated with 0.2 mg PGA day showed no decrease in serum iron concentration nor any reticulocyte response. However with an increase of the PGA dose to 0.5 mg a significant decrease in the serum iron level as well as a simultaneous rise in the reticulocyt count occurred. In the other groups of pernicious anemia treated

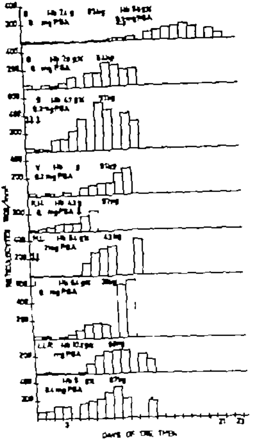


Fig. 5. The reticulocyte response in megaloblastic anemia due to folic acid-deficiency treated with small doses of PGA. Arrows indicate blood transfusions.

with small doses of B_{12} or oral folic acid respectively and in the group of megaloblastic anemia due to folic acid deficiency treated with "physiologic" doses of PGA, the expected drop in serum iron concentration has been observed.

The TIBC values were low before therapy. After 8—10 days of therapy with small doses of either PGA or vitamin B_{12} the TIBC values did not change significantly in most of the cases examined. Twenty days after commencement of therapy the

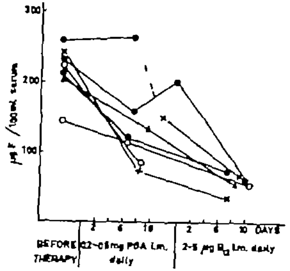


Fig 6. The serum iron concentration in pernicious anemia before therapy and during treatment with small doses of PGA and subsequently B_{12}.
PGA administered a day ▲ 1 µg/kg, ● 3 µg/kg, ○ 5 µg/kg × 7 µg/kg
The broken line indicates change of therapy from 3 µg PGA to 7 µg PGA/kg a day

TIBC rose to normal values in almost all cases irrespective of the kind of therapy given.

Bone marrow morphology

The 5 cases of pernicious anemia (group I b) who were initially treated with 3 µg B_{12} a day all showed a normoblastic erythropoiesis when examined on the 8th day of therapy which coincided with their reticulocyte peak. In the myelopoiesis only a few giant metamyelocytes were found in most cases — in one case (L. B.) they amounted to 2 %. The hemosiderin content was unchanged but the sideroblast count decreased considerably. In 2 cases it was in the iron-deficiency range, indicating increased hemoglobin production (27). In the patients with pernicious anemia who were treated with oral PGA in doses of 1 mg or 3 mg a day (group I c) both erythro- and myelopoiesis became normal.

Six patients with pernicious anemia initially treated with small doses of PGA (group I a) were reexamined after one to two weeks of therapy and showed only some amelioration of the megaloblastic picture. Those who were totally megaloblastic became partially megaloblastic, the partially megaloblastic ones had only intermediate megaloblasts at the control study. Only slight changes had occurred in the myelopoiesis and in 3 cases the number of giant myelocytes was unchanged. The hemosiderin and sideroblasts were unchanged. Thus, there was a clear-cut difference between small doses of PGA and B_{12} in their effects on bone marrow morphology in patients with pernicious anemia.

In megaloblastic anemia due to folic acid deficiency the bone marrow morphology after therapy with small doses of PGA could be evaluated in 7 cases. In 5 cases the reexamination was done when or just after the reticulocyte peak had occurred. In all of these cases the erythropoiesis had become normal and the myelopoiesis almost normal. Two cases (M. L. and I. J.) who were examined 2—3 days before the reticulocyte peak was reached showed only a moderate amelioration of the erythro- and myelopoiesis.

Haptoglobin and bilirubin levels

Changes in haptoglobin and bilirubin values during treatment were compared between the group of pernicious anemia treated with small doses of B_{12}, and that treated with "physiologic" doses of folic acid and also the group of primary folic acid-deficiency anemia treated with "physiologic" doses of folic acid. The time elapsing until a normal haptoglobin value was obtained varied considerably within each group making definite

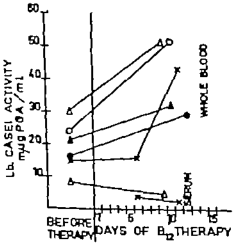


Fig. 7 Whole blood and serum folic acid concentrations in pernicious anemia before and during treatment with small doses of B_{12}.

conclusions impossible. However of the patients with pernicious anemia treated with small doses of B_{12} (group I b) two developed a normal haptoglobin level, three increased the haptoglobin value somewhat and three failed to show any reaction during —12 days of therapy Patients with primary folic acid-deficiency anemia treated with small doses of folic acid reacted in a similar way the haptoglobin level became normal in four increased in two and did not change in two during the same time of observation. However in the group of pernicious anemia initially treated with small doses of folic acid, none of the six examined showed any change in the haptoglobin value during 7—12 days of treatment. Nor did the bilirubin values decrease in patients with pernicious anemia treated with small doses of folic acid.

Vitamin B_{12} in serum

Patients with pernicious anemia treated with physiologic" doses of folic acid showed no rise in the serum B_{12} concentration after 7—14 days of therapy With small doses of B_{12} the serum B_{12} levels became normal in 8 cases but remained subnormal in 3 cases examined after 8—11 days of treatment.

Three of 4 cases with folic acid deficiency initially treated with small doses of folic acid showed a rise in serum B_{12} concentration. In 2 of these cases the increase was possibly due to ameliorated nutrition.

Folic acid levels in whole blood and serum

Pernicious anemia initially treated with small doses of B_{12} showed a considerable rise in *whole-blood* folic acid after 8—10 days of therapy in spite of the fact that the patients were put on a folic acid-deficient diet (fig 7) This rise in L. casei activity in whole blood occurred simultaneously with the reticulocyte peak. One patient who was examined on the 5th day of therapy when the reticulocytes had just begun to increase showed no change in the whole blood folic acid concentration, but 5 days later when the reticulocyte peak was passed the folic acid concentration reached the median normal value (case Ch. M.) In contrast the folic acid concentration in serum decreased somewhat during B_{12} treatment in 2 patients examined.

The same phenomenon was observed in the group of pernicious anemia initially treated with physiologic doses" of PGA and subsequently with small doses of B_{12}. A moderate rise of L. casei activity values was obtained in whole blood as well as in serum during the PGA treatment (fig 8) However after a similar period of B_{12} treatment a steep increase in whole-blood activity occurred whereas at the same time the serum folic acid activity tended to fall.

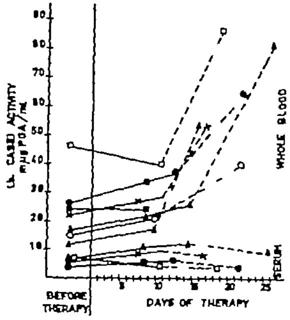


Fig. 8. Whole blood and serum folic acid concentrations in pernicious anemia before and during treatment with small doses of PGA (solid lines) and during subsequent treatment with small doses of B_{12} (broken lines)

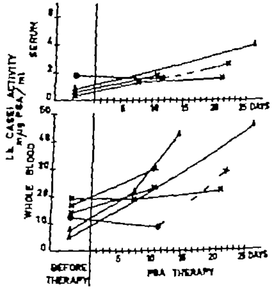


Fig. 9. Whole blood and serum folic acid concentrations in cases of megaloblastic anemia due to folic acid deficiency before and during treatment with small doses of PGA.

Three cases of primary folic acid-deficiency anemia treated with small doses of B_{12} did not show any changes of folic acid activity either in whole blood or in serum. Six patients with folic acid-deficiency anemia treated with small doses of PGA showed a rather small rise in folic acid activity in whole blood in spite of a good reticulocyte response (fig 9) The *serum* folic acid activity of these patients remained in the deficiency range during this therapy and one case (M. L.) had low values even after 18 days of treatment. In two cases (S P and L. R.) who received the largest doses of PGA used in this study (i. e. 0 4 mg a day) the whole blood folic acid activity increased appreciably The serum folic acid activity became also normal in the case examined.

FIGlu excretion

FIGlu excretion in the urine was examined in 12 cases of pernicious anemia before and during the course of therapy The results are seen from table II. Nine patients had initially been treated with small doses of PGA. Five of them had normal or slightly increased FIGlu excretion values and 4 considerably increased FIGlu values. Two of the last mentioned who had the highest FIGlu values had not diminished FIGlu excretion with small doses of PGA on the 8th or the 22nd day of therapy respectively The FIGlu excretion in case G K. diminished successively during subsequent therapy with small doses of B_{12}, but still had pathologic values after 2 weeks of treatment. After further 10 days treatment with 100 μg B_{12} a day the FIGlu excretion value decreased but was still above normal. In case HJ J the FIGlu excretion remained abnormal after a subsequent 6 days of therapy with 3 μg B_{12}, but became normal after 2 days of treatment with 1 000 μg B_{12}.

Table II. Studies of FIGlu excretion in patients with pernicious anemia initially treated with small doses of PGA and subsequently with small and large doses of B_{12}, and in the group of pernicious anemia initially treated with small doses of B_{12}

Subject	FIGlu excretion before therapy μM/hr	PGA therapy		FIGlu excretion		B_{12} therapy		FIGlu excretion	
		Dose mg	Days	Day*	μM/hr	Dose μg	Days	Day*	μM/hr
O.K.		0.2	1—6	2	245	5	12—23	17	183
		0.5	7—11	8	195			21	120
						100	24—34	28	116
								34	37
H.J.		0.2	1—27	7	127	2	16—27	22	104
				13	71	1,000	28—30	30	19
F.O.	63	0.5	1—10	6	40	3	11—19	15	19
						1,000	20—21	22	10
L.B.	61	0.2	1—9	1	77	3	10—20		
				3	60	100	21—30	31	8
Ax.K.	32	3	1—11	1	23				
	29			2	16				
	24			3	16				
A.B.	24	0.4	1—10	1	19				
	14			3	15				
	20			8	16				
				10	12				
J.F.	11	0.4	1—8	2	10				
	19			5	9				
V.A.	16	0.5	1—7	2	6				
				3	4				
A.K.	10	0.4	1—9	1	14				
	3			2	11				
				3	3				
Ch.M.	17								
	17					3.5	1—11	1	28
								2	27
L.B.								3	30
	3								
	5					3	1—13	1	2
	5							2	2
E.J.								3	<1
	3								
	3					3	1—11	1	6
	6							2	4

Duration of therapy at the dose stated, reckoned from the first day of therapy
Day of therapy on which the FIGlu estimation was done.
Single load of 15 g l-histidine.

Table III FIGlu excretion in folic acid deficiency anemia

Subject	FIGlu excretion before therapy μM/hr	B_{12} therapy		FIGlu excretion		PGA therapy		FIGlu excretion	
		Dose μg	Days	Day[a]	μM/hr	Dose mg	Days	Day[a]	μM/hr
K. J.[a]		1 000	1–7			0.2	28–39	37	140
		50	8–27	22	340	15	40–42	43	4
A. S.	67	2.5	1–7	7	224	0.2	12–21	18	70
		5	8–11						
S. P.	18	5	1–8			0.4	14–26	14	29
	13	100	9–13	13	16			15	38
								16	4
K. H.		1.5	1–6			0.2	11–15	12	1
		50	7–10	8	48			13	0
				9	43				
				10	92				
I. J.	840					0.2	1–9	6	310
								8	260
						15	10–13	13	13
G. A.	76					0.1	1–10		
						0.2	11–21	22	90
						15	22–29	31	19
G. F.	39					0.1	1–13	14	14

For explanations see table II.

Seven cases with folic acid-deficiency anemia were studied (table III). Four of them initially treated with B_{12} in small or pharmacologic doses showed either no change or somewhat increased FIGlu excretion after 7—22 days of therapy. In three cases with slightly increased FIGlu excretion it became normal after 2—14 days of treatment with small doses of PGA. The other cases except G. A. with high excretion values showed a considerable fall in the FIGlu excretion but were not normal after 6—9 days of treatment with "physiologic" doses PGA. After treatment with 15 mg PGA for 2 or 3 days respectively their FIGlu values became normal.

Discussion

It is evident from the results that "physiologic" doses of folic acid had a different effect upon hematopoiesis and other parameters in primary folic acid deficiency than in Addisonian pernicious anemia. All cases of folic acid deficiency responded to small doses of PGA with an adequate reticulocyte peak, whereas the reticulocyte peak in the group of pernicious anemia never reached the expected mean response value. No overlapping in the reticulocyte response between the two groups was observed. However 4 of the patients with pernicious anemia reacted with a significant rise in the reticulocyte number while on 0.2—0.4 mg PGA a day

In two of them the reticulocyte response was highly impressive (fig 2) As the reticulocyte response in megaloblastic anemia is dependent on the initial red cell count, bone marrow activity and complicating diseases, such reticulocyte responses might in some patients be at the lower limit of a reticulocyte response to PGA therapy in primary folic acid-deficiency anemia (case G A.) This uncertainty in the therapeutic trial can often be eliminated if the PGA therapy is followed by small doses of B_1 (fig 1) The secondary reticulocyte peak obtained in all cases but one (case A. B.) during subsequent therapy with small doses of B_{12} far exceeded the former one, indicating that the response to PGA therapy was inadequate for the given individual. It is suggestive that 0.4 mg PGA i. m. a day even for men with a high body weight is a dose too large as to be used for a diagnostic-therapeutic trial (case A. B. fig 2) For this purpose t is important to determine the lowest dose of PGA that would give an unequivocal reticulocyte peak in all cases of folic acid deficiency anemia within 10—12 days of therapy It must further be assessed whether such a dose would leave the patient with Addisonian anemia without a reticulocyte response. It is suggestive that with diminution of the PGA dose the risk for overlapping in the reticulocyte response might be abolished. In our material 7 patients with primary folic acid deficiency anemia in different ages and body weights have had an optimal reticulocyte response to 0.2 mg PGA or less. Two of 3 patients who have been treated with 0 1 mg PGA day responded with an adequate reticulocyte peak. The third patient (G A.) with a body weight of 85 kg did not respond but answered with a satisfactory reticulocyte peak when the dosage was increased to 0.2 mg PGA a day Sheehy et al. (28) found that many patients with megaloblastic anemia in tropical sprue responded to 25 μg PGA orally a day however some needed 150—250 μg PGA parenterally Some of their patients who did not respond to the minute" doses PGA had extremely low B_{12} values. In our case the B_1 value in serum was rather high-normal. For the diagnostic trial the dose of 0.2 mg PGA a day should thus not be exceeded. It seems possible that 2 μg PGA per kg body weight will suffice to give a diagnostic reticulocyte response within 10—12 days in all cases of megaloblastic anemia where folic acid deficiency is the only limiting factor

If pernicious anemia is suspected on clinical grounds the diagnostic trial should be initiated with 3 μg B_1 i. m. a day The reticulocyte response is not delayed by more than 2—3 days as compared with pharmacologic doses. It may be difficult to ccomplish the therapeutical trial in severe anemia without giving blood transfusions. The supply of packed red cells does not seem to interfere with the reticulocyte response if an appreciable anemia remains after transfusion.

During the trial the caloric intake should be limited and food rich in folic acid excluded from the diet in order to avoid spontaneous remissions as pointed out by Jandl and Lear (10) This is of especial importance when patients with megaloblastic anemia due to nutritional deficiency or cirrhosis of the liver ar studied.

Ungley (26) found that the red-cell rise after 10 days of therapy is a useful criterion of the potency of a B_{12} preparation when administered in a single small dose to patients with pernicious anemia. In all

our patients with pernicious anemia treated with small doses of B_{12}, as well as in cases with folic acid-deficiency anemia treated with small doses of PGA, the red cell count increased significantly during 10 days of observation However none of the cases with pernicious anemia treated with "physiologic doses of PGA, except case A. B discussed above, showed any rise in the red cell count during a similar time of observation. Corresponding dissimilarities were seen in the bone marrow morphology when the two groups of megaloblastic anemia were treated with "physiologic" doses of PGA.

The fall in serum iron concentration, otherwise regarded as a useful index of the efficacy of treatment in megaloblastic anemia, did not show any essential difference between the two kinds of megaloblastic anemia when both were treated with small doses of PGA. The serum iron concentration diminished in both. However the sideroblast count in the bone marrow smears dropped in folic acid deficiency anemia but remained unchanged in pernicious anemia on PGA therapy This may be explained by the fact that the *serum* iron pool is very small and sensitive even to the minute increase in the erythropoietic activity caused by small doses of PGA in pernicious anemia.

Our haptoglobin studies suggest that a different reaction concerning the hemolytic state exists in folic acid-deficiency anemia and pernicious anemia during short term treatment with small doses of PGA. However the variations within the groups were so large as to deprive the haptoglobin examination of a differential diagnostic value.

Serum B_{12} concentrations in patients with pernicious anemia did not change during therapy with small doses of PGA. They rose quickly after treatment with 2—5 μg B_{12} a day but seldom reached the mean value after about 10 days of therapy Six cases of folic acid-dependent megaloblastic anemia had decreased B_{12} concentrations in serum. Two of the five examined had a normal Schilling test. During treatment with small doses PGA, 3 of the 4 cases studied showed an increased B_{12} concentration, in 2 cases possibly due to ameliorated nutrition. In a recent paper Sheehy et al. (29) reported that the serum B_{12} levels tend to increase in patients with tropical sprue after long term therapy with minute doses of PGA orally

Most of the cases with pernicious anemia in our series who did not get blood transfusions before examination had low whole blood folic acid levels and normal or rather high serum folic acid levels (table I) After 8—10 days of treatment with small doses of B_{12}, in spite of a folic acid-deficient diet, the whole blood folic acid values increased considerably and the serum folic acid levels decreased. This rise in whole-blood folic acid level coincided with the reticulocyte peak obtained during therapy and remained at the same level on further examination. This rapid increase in red cell folic acid content indicates that the folic acid stores were not empty in spite of low values before therapy Cases of pernicious anemia treated with small doses of PGA increased only slightly in whole-blood folic acid concentration but showed a striking increase in red-cell folic acid when subsequently treated with small doses of B_{12} (fig 8) Apparently the increase in red-cell folic acid was not caused by passive diffusion. Our results suggest that B_{12} deficiency may interfere with folic acid incorporation into erythroblasts and might be the cause of low folic acid content in whole blood in cases of

pernicious anemia. The low whole-blood folic acid levels in pernicious anemia are often associated with a high-normal folic acid level in serum and hence the serum folic acid examinations seem to be of superior diagnostic value in megaloblastic anemia (30). However it is notable that those patients with pernicious anemia who responded with a slight reticulocyte increase to small doses of PGA had the lowest whole-blood folic acid levels but normal serum activity. In folic acid-deficiency anemia treated with small doses of PGA, the folic acid concentrations in whole blood and in serum examined after the reticulocyte peak has been reached remained somewhat low in most cases, indicating emptied folic acid stores.

High FIGlu excretion values were formerly regarded as diagnostic for folic acid-deficiency (31); however Silverman and Pitney (32) in rats and Fox et al. (33) in chickens have shown that B_{12} deficiency gives rise to large amounts of FIGlu in the urine. Zalusky and Herbert 1961 (34) have reported high FIGlu values in 2 cases of pure B_{12} deficiency. Our results support the above findings. High FIGlu excretion values in pernicious anemia persisted or increased during therapy with small doses of PGA. On the contrary in folic acid-deficiency anemia a decrease in the FIGlu excretion was seen in all uncomplicated cases during similar treatment, indicating that the cause of the impaired histidine metabolism is different in pernicious anemia and folic acid deficiency. The high FIGlu excretion in folic acid-deficiency anemia did not improve on high and prolonged B_{12} therapy. In pernicious anemia ameliorated FIGlu excretion was seen even with small doses of B_{12}. The FIGlu test after large doses of PGA does not possess any differential diagnostic value.

Diagnostic trials with small doses of PGA in megaloblastic anemias have made prominent the question of how much folic acid may be given without endangering patients with unrecognized pernicious anemia. Marshall and Jandl (9) suggested that a dose of 0.2—0.5 mg PGA orally would suffice to prevent dietary deficiency of folic acid and be safe in B_{12} deficiency. However the failing response to 0.4 mg PGA a day in the short-term trial in pernicious anemia does not bear out the question of the safety of this dose during long term treatment. In fact Baldwin and Dalessio (13) reported spinal cord degeneration but a normal hemoglobin value in a patient who had been treated for 16 months with a multivitamin preparation containing a daily dose of 0.5 mg PGA. Our results for short-term treatment of pernicious anemia with PGA clearly show that an oral dose of 1 mg PGA a day would rapidly normalize the hematopoiesis in pernicious anemia. Similar results have been reported by Ellison (11) and Crosby (12) in long term treatment with multivitamin preparations containing folic acid. In case A. B. a normal bone marrow and an increase in hemoglobin was obtained after short-term treatment with only 0.4 mg PGA parenterally. With smaller doses, down to 0.2 mg PGA i.m., some reactions were seen during short term therapy. As oral PGA seems to be utilized as effectively as parenteral PGA, we consider that 0.4 mg PGA orally is dangerous in prolonged treatment. Since the question of how much PGA can be given in pernicious anemia during long term therapy without masking the disease cannot be answered as yet, the discussion of daily allowances of PGA in multivitamin preparations must be limited to the consideration of the amounts known to be

unsafe in pernicious anemia and the amounts required to prevent nutritional folic acid deficiency In a recent report by Mangay Chung et al. (35) authorized by the A. M A. Council on Food and Nutrition a "high cost diet was found to contain about 190 μg "total folic acid a day The meals were prepared according to accepted home cooking procedure. Sheehy et al. (28) has shown that a hematological and clinical remission can be obtained in many cases of tropical sprue with as little as 25 μg PGA orally a day Our cases of folic acid-deficiency anemia of varied etiology responded rapidly to 100—200 μg PGA a day These data indicate that 100 μg PGA a day as a supplement to the diet is sufficient to prevent nutritional folic acid deficiency Thus there are no grounds for allowing more than 100 μg PGA a day in multivitamin preparations sold without prescription to healthy people. This dose has hitherto not given any reaction in the acute trial in patients with pernicious anemia.

Summary

The effect of small doses of folic acid and B_{12} on different hematological and biochemical parameters were studied in 26 patients with megaloblastic anemia, 9 of the cases being due to folic acid deficiency The doses of folic acid used were 0 1—0 4 mg pteroylglutamic acid (PGA) i. m. daily and those of B_{12} 2—5 μg i. m. a day

An apparently different *reticulocyte response* was obtained in the two kinds of megaloblastic anemia with the doses of PGA used All patients with *folic acid dependent anemia* reacted optimally to small doses of PGA (0 2 mg a day) In *pernicious anemia* a slight to moderate response was seen in some cases, especially in those who received 0.3—0.4 mg PGA a day However all cases of pernicious anemia responded with a secondary optimal reticulocyte peak when put on small doses of B_{12} (3 μg a day) immediately after PGA therapy As all patients with folic acid-deficiency anemia responded adequately to 0.2 mg PGA within 10—12 days, we consider that this should not be exceeded for diagnostic purposes even in patients of high body weight. During the diagnostic trial the amount of calories should be limited and food rich in folic acid should be excluded from the diet, especially in cases of megaloblastic anemia suspected to be due to nutritional deficiency Transfusion of packed red cells does not interfere with the diagnostic trial provided that anemia persists sufficiently to give an adequate reticulocyte response.

The bone marrow picture became almost normal in cases of pernicious anemia treated with small doses of B_{12} and in cases with folic acid-deficiency anemia after 8—10 days of therapy with small doses of PGA. Some amelioration of the erythropoiesis corresponding to the slight reticulocyte response was observed even in cases of pernicious anemia treated with small doses of PGA.

Serum iron examined on the 7th day of therapy diminished in pernicious anemia treated with small doses of either B_{12} or PGA, indicating that some change in erythropoietic activity takes place during PGA treatment.

Changes in *haptoglobin total iron-binding capacity depot iron* and *serum B_{12} levels* during therapy are discussed

Despite a folic acid-deficient diet the folic acid levels in whole blood increased considerably during treatment of pernicious anemia with vitamin B_{12},

indicating the presence of folic acid stores. A need of vitamin B_{12} for the incorporation of folic acid into erythroblasts is suggested.

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Wilson's Disease

By

Arne Nordöy

Hepatolenticular degeneration (Wilson's disease) is caused by an autosomal inherited defect (6) with inability to synthesize a normal ceruloplasmin, the plasma copper protein (1). This is a bluish a_2-globulin containing 8 atoms of copper per molecule and of molecular weight 151000 (5). The normal plasma concentration is about 30 mg/100 ml. The exact function of ceruloplasmin is not known.

Experiments with radioactive copper (Cu^{64}) have shown that the major part after intestinal absorption is temporarily incorporated into the liver copper protein (CaLP) (4). From this store about 0.5 mg Cu in 24 hours is transferred into ceruloplasmin. Ceruloplasmin contains about 95 % of the total plasma copper, the remaining 5 % being loosely bound to the serum albumin (3).

Latent or manifest Wilson's disease exhibits four characteristic abnormalities: 1. Partial or complete deficiency of ceruloplasmin (1,2,6), the total plasma copper being decreased with a few exceptions (7, 6). 2. Increased non-ceruloplasmin copper. 3. Increased copper content of many organs, especially the liver, brain and kidneys. 4. Increased excretion of copper in the urine.

Homozygotes develop symptoms of basal ganglia disorder, hepatic cirrhosis, Kayser-Fleischer rings in the corneae, and tubular and glomerular renal dysfunction. Some cases have multiple minor fractures, and bluish lunulae at the bases of the nails are found in some 20 % of the cases (8).

All these symptoms seem to be closely related to abnormal copper deposits. One clinical type shows mainly progressive lenticular degeneration which starts in childhood or early youth and progresses rapidly with a fatal outcome within months. A second type with predominantly hepatic and renal involvement is seen in middle-aged patients and has a more protracted course.

In the family studied, 10 cases had died from lenticular degeneration — one case presented a manifest clinical syndrome, whereas 14 cases showed a reduced ceruloplasmin content in plasma, but without clinical symptoms.

Submitted for publication March 28, 1962.

Table I Hematological findings and liver function tests

Hemoglobin	15.5 g/100 ml
Red cell count	4.65 mill./mm
White cell count	4,400/mm³
Trombocyte count	27,500/mm³
Sedimentation rate	12 mm/h
Total serum protein	5.8 g/100 ml
Serum albumin	2.5 g/100 ml
Serum γ-globulin	2.0 g/100 ml
Prothrombin-proconvertin	46 % of normal
Alkaline phosphatase	2.0 Bodansky U
Bromsulphalein retention	17 % after 45 min
Serum glutamic oxalacetic transaminase	45 U
Thymol turbidity	0.22 extinction (upper normal limit 0.17)

Case report

A 33-year-old man was admitted to the Department of medicine in September 1961 because of tremor and abdominal distress.

He had been in good health until 1954 when he suddenly had melena and was admitted to another hospital. No lesion was disclosed in the gastro-intestinal tract. A vitamin K-resistant hypoprothrombinemia of 40—50 % (P P method) was found, indicating liver disease, but all other liver tests were normal. During the following years he developed pigmentation of the legs, tremor of the head and right hand and also, in the last months before admission, abdominal distress.

Physical examination revealed spider naevi on the upper part of thorax. The liver could not be felt, but he had an enlarged spleen. He showed titubation of the head tremor hyperreflexia and retardation of the active movements of the right upper extremity typical Kayser Fleischer rings and pigmentation of the skin on the legs.

The urinanalysis was negative except for urobilinuria. Results of laboratory tests are given in table I. An X-ray picture of the esophagus showed no varices.

For the verification of the diagnosis of Wilson's disease the investigations shown in table II were carried out. (These determinations were performed by Dr O Skaug at the Central laboratory Lier Mental Hospital.) The serum ceruloplasmin determination was confirmed by column chromatography Determination of amino acids in the urine by paper chromatography showed increased excretion of glycine, serine, glutamine, alanine and threonine. (The determination was performed by Dr P Björnstad at the Central laboratory Rikshospitalet.)

Table II Studies of copper metabolism

Total serum copper concentration	87 μg/100 ml (90—120)
Serum ceruloplasmin	0 U. oxidase activity (6—12)
Urinary copper excretion	485 μg/100 ml (3.5—324)

Normal values in brackets.

On an ordinary diet the urinary excretion of calcium was 310 mg/24 hrs, of phosphorus 1,245 mg/24 hrs and of uric acid 860 mg/24 hrs.

The peroxidase reaction in the leukocytes was positive.

Treatment

The influence of three chelating agents on the urinary copper excretion was first investigated (fig 1) BAL and penicillamine have been used before. Desferrioxamin is a new drug which has been tried as an iron chelating agent. BAL and desferrioxamin gave no significant increase in the copper excretion. Penicillamine gave a maximal urinary copper excretion on a daily dosage of 1 45 g with an average excretion of 3 mg/24 hrs. Our patient was treated as follows

I A diet which contained less than one mg of copper a day (15)

a) Special attention was paid to the water The water in the patient's home was analyzed for copper and found to contain between 0.33 and less than 0 05 mg copper/l depending on when the water was taken. This was explained when we found that the last 50 cm of the waterpipe was made of copper

b) A high protein diet was preferred to promote excretion of copper bound to amino acids in the urine.

2. Drug therapy

a). Potassium sulfide 40 mg orally in capsules with each meal was used (2). Any dietary copper is thereby rendered *insoluble* and excreted via the feces.

b). D-penicillamine (dl-methyl cysteine) in dosage of 150-300-150-300 mg daily orally on empty stomach. The dosage was adjusted according to the urinary excretion of copper. If this amount was less than one mg in 24 hrs the dosage was increased.

No toxicity of the therapy was noted. There occurred remarkable and definite clinical improvement in four months with regress of the neurological symptoms. The titubation of the head disappeared completely and the tremor and ataxia of *his right upper extremity* improved. He regained working ability and was able to write by hand which he had not done for some months.

Liver function tests showed some improvement (table III).

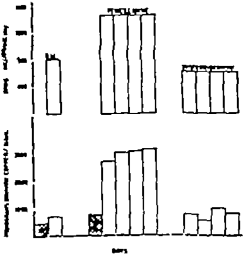


Fig. 1 The cupruretic response of three different drugs.
☐ = control days.

Family history

The family pedigree is illustrated in fig. 2. Three brothers and sister of the patient's father had died at an age of 10 to 15 years with *symptoms* of rapidly progressing lenticular degeneration. Two twin brothers of the patient had died at the age of 13 and 14 years with similiar symptoms. Autopsy revealed hepatic cirrhosis in one of the boys. Wilson's disease was further found in three female and

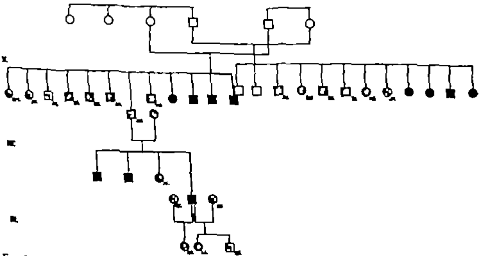


Fig. 2. The pedigree in family with Wilson's disease.
■ Members with Wilson disease.
☐ Members with decreased serum ceruloplasmin level.
☒ Members with normal serum ceruloplasmin level.
☐ Members not examined.

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b) A high protein diet was preferred to promote excretion of copper bound to amino acids in the urine.

(19) in 1956 first reported the use of penicillamine in the treatment of hepatolenticular degeneration, many promising reports have appeared (10 21 22). Side effects are rare (15) and only occasionally is a temporary withdrawal necessary (23 24). In the case reported the improvement of the neurological symptoms was striking. It may take months in some cases before improvement is noted, and in some patients no improvement has been observed (15).

It may be important for the life expectancy of these patients to diagnose the latent stage of the disease and start prophylactic treatment before irreversible tissue damage has developed.

Summary

1. A family of Wilson's disease is reported in which ten members had died with progressive lenticular degeneration. The ceruloplasmin level was reduced in fifteen members of the family.

2. One member only had manifest Wilson's disease with hepatic cirrhosis and with neurologic symptoms for about one year. A striking clinical improvement in four months was observed on a diet low in copper and on therapy with potassium sulfide 40 mg × 3 and penicillamine 0.9 g daily.

3. Penicillamine increased copper excretion in the urine whereas BAL and desferrioxamin had no effect.

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Table III Liver function tests

	24.10. 1961	18.1 1962	19.2. 1962
Bromsulphalein retention %	17	—	8
S-GOT U	45	23	18
Alkaline phosphatase Bodansky U	2.0	3.5	2.0
Prothrombin proconvertin %	46	56	46
Serum protein g/100 ml	5.8	6 1	6.4
Serum albumin g/100 ml	2.5	2.7	3.5
Serum γ-globulin g/100 ml	2.0	1.9	1 4
Thymol turbidity (normal < 0.17)	0.22	0.11	0.12

Table IV Serum ceruloplasmin and total serum copper values in three generations of the patient s family

Subject	Relationship to patient	Serum ceruloplasmin (U oxidase act.)	Total serum copper (μg/ml)
T G	Patient	0	87
O G	First wife	7	118
D G.	Second wife	6	140
O G	Daughter	3.5	120
E. G	Daughter	1.5	—
S. G.	Son	6.5	130
S. G.	Father	4	135
J G.	Mother	2.5	77
A. G.	Father's brother	5	136
F G.	Father s brother	4	106
R. G.	Father's brother	3.5	122
A. G	Father s brother	6	135
T G.	Father's brother	4	—
J N.	Father s sister	7.5	150
R. M.	Father's sister	7.5	112
J N	Father's maternal cousin	6	125
M. S.	Father's maternal cousin	5	147
T H.	Father's maternal cousin	3.5	100
E. H.	Father's maternal cousin	3.5	112
S. H.	Father s maternal cousin	5.0	—
G. Ø.	Father's maternal cousin	3.0	80

one male maternal cousins of the patient's father

The ceruloplasmin and total copper levels in the serum of members of this family are shown in table IV Fourteen of these had a decreased serum ceruloplasmin level. They were all healthy

Discussion

Knowledge is still lacking concerning the nature of the biochemical abnormalities in Wilson s disease and the exact mechanism of the tissue damage — whether it is caused by copper deposition as such or by other factors. The clinical diagnosis of Wilson s disease is based on a history of similar illness in the family characteristic symptoms of hepatic and neurologic disorder Kayser Fleischer rings in the cornea on slit lamp examination, and the specific laboratory findings. Deficiency of normal ceruloplasmin increase in urinary copper excretion and increase in the copper content of the liver (15) are findings which are sufficient to make the diagnosis even if there are no clinical symptoms except in the heterozygous carriers of the disease. Palos (25) reported on a negative peroxidase reaction in the leukocytes as characteristic in Wilson s disease. This observation was not confirmed in our patient.

Administration of ceruloplasmin has been tried without effect (10 11) Stimulation of ceruloplasmin synthesis with estrogens has also been tried, without success exept in one case (12 13 14 26) The therapy should aim at producing a negative copper balance by reducing the copper content in the diet, reducing the absorption of copper from the gut and removing copper from the body The use of chelating agents and a low copper diet will probably make the future of these patients more hopeful. Since Walshe

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The Effect of Common Antacids on Intragastric pH

With Special Reference to Dihydroxy Aluminium Sodium Carbonate (Minicid)

By

Julius M. Marcussen

The evaluation of antacids is usually based on in vitro experiments by means of HCl-solutions or aspirated gastric samples. Titration or pH measurement may give valuable information as to the potential acid binding capacity. Based on such experimental results many of the common antacids have been selected. Adequate doses have been suggested for maintaining pH within the so called physiologic zone (pH between 2.5—4) (5, 11, 16).

Antacids given orally will usually meet a constant flow of highly acid gastric secretion which instantly minimizes the buffering effect. Another important factor is the short time for action. In addition to the hypersecretion most ulcer patients have an increased gastro-duodenal motility. This tends to squeeze the buffering substances through the pylorus causing high pH levels in the antro-duodenal cavity. In vivo experiments are therefore preferable in studying the efficacy of different antacids. The direct biological method seems to attract increasing interest (10, 15, 18, 21).

Material and methods

During the last three years the author has examined commonly used antacids by means of intragastric pH recordings (15). Except for a few healthy controls, patients having active gastro-duodenal ulcers were selected for testing. In the author's experience it is preferable to base the experiments mainly on subjects having a low and nearly constant fasting pH between 1—2. Such cases prove a critical test for antacids. At higher levels of pH fluctuations may occur making difficulties in interpretation. On a total of 29 patients, 36 examinations have been accomplished. Seventeen of the cases (10 men and 7 women) had duodenal and eight (5 men and 3 women) gastric ulcers. Among the 17 males and 12 females the age varied from 17 to 60 years.

As previously published (15), all patients were examined fasting at a specified time in the morning. Two days prior to the examina-

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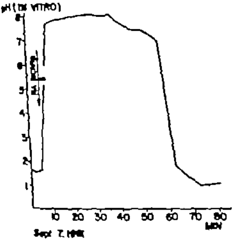


Fig. 3. The fig shows the untoward raise and fall reaction to strong alkalis (5 g $NaHCO_3$). Note the secondary fall of pH far below the initial values.

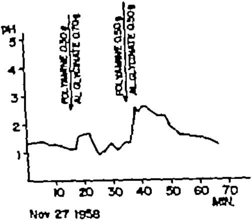


Fig. 4. Polyamine-resins mixed with aluminium glycinate only gives small and transient increases f pH — in fundic readings (S. E., male 52 yrs)

electrode is placed in the fundus of the stomach.

In a third test (female I. H. 51 yrs) a duodenal tube was placed exactly in the angular region (fig. 2). As shown in fig. 3 the buffering effect of the same dose of NaHCO lasted more than 40 min. as found by small, fractionated aspirations. The sudden fall to 1.4—1.06, far below the initial values, is noteworthy.

II. THE EFFECT OF SOME OTHER ANTACIDS

A *mixed antacid* ("Zeolitt comp.") in teaspoon-doses gave small rise of pH from 0.93 to 1.67 for only 12 to 13 min. Tablets of *aluminium hydroxide gel* (Alludrose) in repeated doses had a minimal effect for about 10 min. in a case of hypersecretion (fig. 1).

Glycine-calcium carbonate ("Titralac") was firstly tested on aspirated gastric juice. In this in vitro trial 1 tablet raised the pH from 1.10 to 6.40 within 8 min. In similar test the same dose gave a gradual rise of pH from 1.68 to 6.18 in the course of 7—8 min. A fairly strong in vivo response might therefore be expected. By intragastric measuring at low pH values, the effect of 1 tablet of Titralac was moderate to small and shortlasting. In patients with fasting pH of about 2.5 the effect was more satisfactory but did not last more than 12 to 13 min. (fundic recordings).

Aluminium glycinates were tested separately and mixed with *polyamine resins* (Macrostyrine). The intragastric effect on 6 cases of ulcer disease resulted in moderate to small increases in pH, with duration of 5 to 22 min. The fifth case (male, S. E., 52 yrs) is illustrated in fig. 4 and represents the most satisfactory result. It is assumed that the reaction to the second dose is caused by an additive action. In vitro experiments on polyamino resins ("Macrin") were performed. Doses of 1.2 g were stirred in 50 ml aspirated gastric secretion. The pH rose slowly from 1.12 to 2.39 within 40 min. with continuous stirring. In a strictly

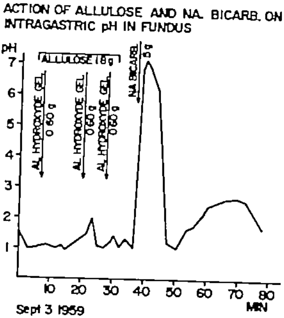


Fig 1 The minimal intragastric effect of aluminium hydroxide gel tablets in contrast to sodium bicarbonate is seen in a case of duodenal ulcer (G M. male, 30 yrs) The electrode tube was kept in the fundic region.



Fig 2. Sept. 7 1961. The X ray taken at the end of the third experiment on $NaHCO_3$ show that the duodenal tube was placed exactly in the angular region (I H female, 51 yrs) The pH of the fractionated aspirations is seen in fig 3.

tions all drugs were withdrawn. The Beckman glass electrode (No 39042) for gastro-intestinal pH measurement was placed in the fundus or in the angular region by fluoroscopic control (see the X rays) Simultaneous readings using 2 electrodes at different levels (fundic + angular) did not succeed. During the experiments all patients were placed in bed in a half recumbent position. The thin covered electrode tube through the nasal passages did not disturb any of the patients. Reference KCl-electrode under the tongue completed the circuit. All patients relaxed satisfactorily as distractions were avoided.

After a control period of 5 to 15 min. with frequent pH readings, the antacid dose was given in a glass of water (150 ml) Then pH readings were performed at 1—2 min. intervals for the first quarter of an hour and during the following hour every 4—5 min. No stimulants were given. The following antacids were tested with the outlined procedure Sodium bicarbonate, silicates, hydroxides, glycinates, glycine-carbonates, polyamine resins and dihydroxy aluminium sodium carbonate (DASC) Most of the patients were given the usual recommended dosage.

Results

I. THE BUFFERING EFFECT OF STRONG ALKALIS (NaHCO)

A dose of 5 g sodium bicarbonate was given to a normal subject (nurse, 19 yrs) The pH rose rapidly from 2 to 7 for about 50 min. followed by a decreasing period of 15 min

The next experiment is illustrated in fig 1 A five g dose of NaHCO resulted in an extremely rapid rise of pH from 1 06 to 6 77 The maximum value of 7 16 was kept for a few minutes. Then a steep fall to 1 22—1 02 occurred in the course of 8 to 12 min. The second pH rise vanished after half an hour The diagram (fig 1) seems to give the typical reaction of sodium bicarbonate when the glass

Fig. 7 a. At angular level, 1 tablet of Minicid (DASC) seems to give an adequate response (L. H., female, 51 yrs). The location of the electrode tube is shown in figs. 7 b and c.

Fig. 7 b. Sept. 6, 1961. The X-ray shows the angular position of the glass electrode, which was connected to Beckman pH meter model G. The pH readings on testing Minicid (DASC) are seen in fig. 7 a.

Fig. 7 c. With small amount of Barium the tip of the glass electrode is readily seen at angular level (compare fig. 7 b). The X-ray was taken after completed Minicid test (see fig. 7)

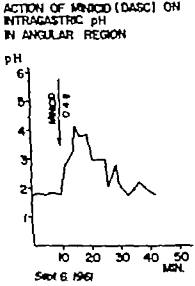


Fig. 7 a.



Fig. 7 b.

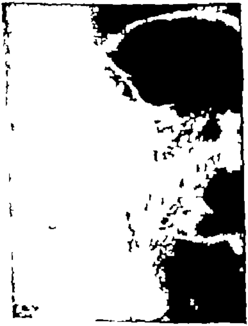


Fig. 7 c.

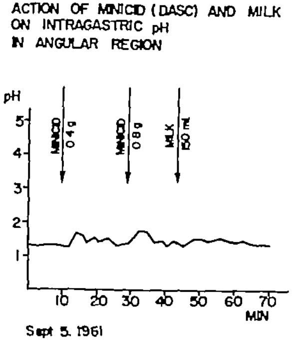


Fig 5. In the exceptional case with great hyper secretion Minicid (DASC) gave small, but distinct raises of pH. In such cases doses ought to be higher 150 ml milk failed to give any pH response (E. M W female, 17 yrs)

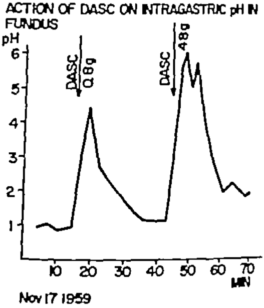


Fig 6. Minicid (DASC) is seen to act satisfactory on intragastric pH. The largest dose of 12 tablets did not bring the pH above 6.10 (K. S male, 21 yrs)

controlled case of gastric ulcer (male, O K. 47 yrs) polyamino-styrene was given for 20 days in a dosage of 10 to 12 g daily divided into small doses of 0 6—0 9 g The histamine response was tested before and during this treatment by continuous suction method (12 13 14) There was no effect either on the pH values (in vitro) or the secretion volumes. Nor was there any reduction of pepsin (Hunt's method) He became, however symptom free and the large gastric ulcer had diminished significantly This case illustrates the problems incurred when antacids are to be evaluated.

The intragastric effect of milk

It is a well known experience that a glass of milk may give prompt relief of pain to ulcer patients. As milk remains a convenient supplement to the diet of dyspeptics it was thought to be of interest to study its intragastric action. It was a surprise to note how little it affected pH intragastrically In two experiments very small and limited changes of pH occurred. The last test is seen in fig 5 After the glass of milk (150 ml) the patient felt better but there was no change in the pH.

III. THE PROPERTIES OF DIHYDROXY ALUMINIUM SODIUM CARBONATE

This relatively new compound named DASC, has been thoroughly tested by several investigators (5—8 10 11 17—20) As the formula $Al(OH)_2 O CO_2Na$ would suggest, this specially prepared antacid (5) possesses many of the properties both of aluminium hydroxide and sodium bicarbonate It neutralizes acid rapidly with release of carbon dioxide, but unlike sodium carbonate does not produce a pH on the alkaline side. DASC also exhibits the prolonged neutralizing power and buffering action of aluminium hydroxide gel.

noted that the author has used higher doses of sodium bicarbonate than other investigators.

In all cases of hypersecretion, especially in duodenal ulceration, *the antacid treatment should be supplemented by an anticholinergic drug.* The reduced secretion volume and motility will give a more favourable condition for the buffering capacity of antacids. This important fact of diminished acid output is illustrated in fig. 8. In a patient with two gastric ulcers and a deformed duodenal bulb (male, S. L., 39 yrs) the extreme hypersecretion by histalog was reduced from 367.5 ml/60 min. to 133 ml/60 min. after a dose of 100 mg Demotil ("Prantal") orally. On the combined antacid and anticholinergic treatment ulcers healed within 3—4 weeks. By intermittent therapy the patient is still, 4 years later free of symptoms.

Summary

1. [illegible] vivo studies of antacids illustrate the untoward effect of sodium bicarbonate with a secondary fall of the intragastric pH below the initial values. This finding is in accordance with experimental work.

2. Different silicates, hydroxides, glycinates, glycine carbonates, ion exchange resins and even milk appeared to have a minor influence only on pH in the stomach.

3. Dihydroxy aluminium sodium carbonate (DASC, Minicid) had a more significant and prolonged effect on the intragastric pH than any of the antacids mentioned above. To the author Minicid seems to be the most favourable drug among those tested.

The importance of supplementing the antacid therapy with anticholinergic compounds is stressed, especially in duodenal ulcer cases.

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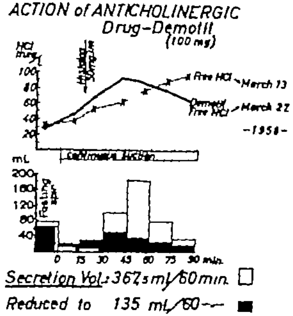


Fig. 8. In a case of extreme hypersecretion histalog stimulated secretion volume was reduced to 1/3 by giving an anticholinergic drug orally (Demotil, 100 mg) (S. L., male 39 yrs.)

The effect of DASC (Minicid Pharmacia) was examined in eleven cases (8 men and 3 women). Seven of the patients had duodenal (3 women) and 4 gastric ulcers. The average age was about 41 years. Except for one case (female, E. M. W. 17 yrs) with extreme hypersecretion due to duodenal ulcer (fig. 5) the intragastric effect of dihydroxy aluminium sodium carbonate was prompt and lasted longer than any of the other antacids tested. As seen in the exceptional case (fig. 5) Minicid in minimal doses gave small, but distinct rises of pH, whereas 150 ml milk had no effect at all. For the remaining 10 cases it was impossible to bring the pH above 6 even with extremely high doses (12 tablets = 4.8 g). Fig. 6 and 7 illustrate the reproducible intragastric pattern of the Minicid effect. The effectiveness seems to be satisfactory even by measurement in the fundic region of the stomach. No untoward reaction like that observed with sodium bicarbonate occurred.

In a clinical study of ulcer patients refractory to ordinary medical treatment, Minicid appeared to have a rapid pain relieving property. In this small group other well known and tested antacids failed.

Discussion

Owing to the powerful neutralizing effect, sodium bicarbonate is still widely used by sufferers from peptic ulcer. The lowering of intragastric pH below the initial values in the second phase is unfavourable to ulcer patients. This reaction to strong alkalis is quite in accordance with results obtained in experimental work on dogs and gastroenterostomized human subjects (1–4). It has been clearly demonstrated that a rise of pH in the antro-duodenal region greatly stimulates acid gastric secretion. This may explain the rapid pH fall seen in figs. 1 and 3, especially with the tube placed in the fundic region. It is evident that less potent alkalis are to be preferred in the medical treatment of peptic ulcer.

The potency of commonly used antacids tested is questionable in cases of active ulcers with hypersecretion. Ion exchange resins seem to fail by the in vivo method. The favourable and distinct pain relieving effect of milk does not seem to be due to neutralizing capacity.

In animal experiments dihydroxy aluminium sodium carbonate (DASC, Minicid) proved to be the most effective antiulcer antacid (6, 7, 9, 19). In vivo studies on human subjects have established that DASC has both prompt and prolonged acid neutralizing properties (8, —10, 17, 18). By the intragastric method antacids in decreasing order of their effectiveness were found to be: DASC, aluminium hydroxide, calcium carbonate and sodium bicarbonate. It has to be

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From the Department of Medicine (Head H. Malmros, M. D.)
University Hospital, Lund, Sweden

Separation of Lymphocytes from Peripheral Blood by Means of a Glass Wool Column

A Method for in vitro Culture of Lymphocytes

By

L. Brandt, J. Börjeson, A. Nordén and I. Olsson

The observation by Nowell (12) that leukocytes from the peripheral blood undergo mitosis in in vitro culture in the presence of phytohemagglutinin has made it possible to obtain ample material for chromosome studies. It has also resulted in valuable genetic information hardly obtainable by other methods. The question that immediately presented itself to hematologists was which type of cells is responsible for the mitosis.

Studies with H³ labelled thymidine (1, 2, 15) have shown that circulating mononuclear leukocytes are capable of division. The polymorphonuclear leukocytes show rapid degeneration in vitro and are therefore probably not able to undergo mitosis in culture (5, 10). It is a commonplace that the rate of mitoses in cultures from peripheral blood containing many polymorphonuclear leukocytes is lower than in cultures of blood poorer in polymorphonuclear leukocytes.

Supported by grants from Riksföreningen mot Cancer, L. Dedeyers' donationsfond and Hanna Landby fund which is gratefully acknowledged.

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The present report describes a method for the rapid production of reasonably pure suspensions of lymphocytes for culture. It utilizes the observation of Johnson and Garvin (8) that polymorphonuclear cells tend to adhere to glass wool.

Material and methods

Glass wool (Pyrex fiber No. 5030, Corning Glass Works, Corning, New York) was packed in a glass tube 3 cm in diameter. In the bottom of the tube was an opening 0.5 cm in diameter. The glass wool was compressed for 12 hours with a rubber stopper. The volume of glass wool used was such that after compression it gave a column of a final height of 3 cm. The column was sterilized at 180° C for 2 hours. During the early part of the work the glass wool and the tube were siliconized, a step that later proved unnecessary.

Twenty ml of heparinized venous blood was withdrawn and immediately poured onto the glass wool column. Gentle pressure was applied by means of gas flowing from a tube containing a mixture of 5 % carbon dioxide, 10 % oxygen and 85 % nitrogen. The pressure was adjusted to give a blood flow of about 0.5 ml/min. The whole procedure was performed in an incubator at 37° C as the

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Table I Percentage of mononuclear cells in blood filtrate and percentage of cells in mitosis

Age (males)	Mononuclear cells %	Cells in mitosis %
26	88	12
27	97	15
28	85	16
23	92	16
46	95	10
26	92	9
23	92	11
45	94	11
47	96	10
26	100	12

adhesiveness of the polymorphonuclear leukocytes to glass is greatest between 25 and 44 C (6) The filtered blood was collected in a test tube and 2 ml of 6 % dextran (Macrodex, Pharmacia, Uppsala) was added. The tube was left at room temperature for 15 minutes. The supernatant cell suspension was removed, care being taken to avoid contamination with erythrocytes. The cell suspension was centrifuged at 200 for 5 min. at room temperature. The supernatant was discarded and the sedimented cells were suspended in culture medium The culture medium consisted of Parker 199 (seven parts) and human serum (three parts) to which had been added 200 U of penicillin and 100 μg of streptomycin per ml. The number of leukocytes in the culture medium was counted in a Buerker counting chamber and adjusted to around 800 000/ml A smear was prepared from the suspension and stained for differential counting

Aliquots (2 ml) of the cell culture medium suspension was added to culture tubes. To each tube was added 0 1 ml of a water extract prepared from *Phaseolus vulgaris* In preliminary experiments a 1 512 dilution of this extract had proved to stimulate the formation of mitosis. A dose of colchicine — 0.2 ml of a 10^{-4} M solution — was also added to each tube. The tubes were incubated at 37 C for 72 hours. The cells were then subjected to hypotonic treatment by the addition of 3 ml distilled water to each tube and incubation at 37° C for 7 min. The cell suspension was centrifuged at 120 g for 5 min., and the supernatant was removed. The sediment was fixed by treatment for 30 min. in 1 ml of a mixture of 10 N acetic acid and 0.1 N hydrochloric acid The cell suspension was centrifuged again, the supernatant discarded and a few drops of orcein dissolved in 10 N acetic acid were added. The cells were suspended in the orcein solution and a small drop of the suspension was placed on a microscopic slide and covered with a cover glass. Gentle pressure was applied to the cover glass to squash the cells. The number of cells in metaphase per 1 000 cells was recorded.

Results

Table I gives the results of 10 separation experiments. The mononuclear cells represent between 88 and 100 % of the leukocytes in the filtered blood. After 72 hours culture these cells showed a mitotic frequency of 90 to 160 per 1 000 cells. This number invariably exceeded the number of polymorphonuclear cells in the filtered blood The presence of colchicine in the culture medium excluded the possibility of the counts including second or later generations of cells It therefore sounds most likely that the cells undergoing mitosis were the mononuclear cells. Cells filtered in the way described give very satisfactory mitosis for chromosome analysis (fig 1)

Discussion

Methods for the preparation of pure leukocyte suspensions are in great demand for many types of studies (11) Most methods utilize centrifugation (7 9) Cassen et al. (4) used a magnet to remove granulocytes which had phagocytized iron This method was used by Carstairs (3) for the culture of lymphocytes. The separation of the leukocytes by filtration through glass wool is a time-saving method which may be of considerable importance in studies of living

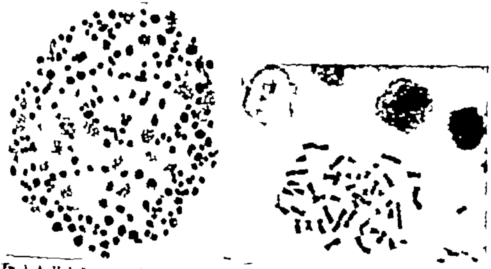


Fig. 1. A. High frequency of mitoses (× 100) B. Chromosomes (× 1,000)

cells. The morphology remains unchanged. The number of mitosis is rather high in in vitro culture, which may also be taken as an indicator of the absence of any damage.

Summary

A method for the separation of lymphocytes from the peripheral blood for culture studies is described, which utilizes glass wool for the adsorption of the granulocytes as described by Johnson and Garvin (8). Heparinized blood is passed through a glass wool column under slight pressure at 37 C. The filtrate gives a lymphocyte content of 88 to 100 % of the filtered leukocytes. In the presence of phytohemagglutinin cultures produced between 90 and 160 mitoses per 1 000 cells. The quality of the mitotic figures is satisfactory for chromosome studies.

The findings support the view that it is the lymphocytes that are responsible for the mitoses in cultures of peripheral blood.

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Local Reaction to Epinephrine Injected Intradermally in Man — a Sign of Endotoxinaemia?

By

Hans Fritz, Karl Erik Hagstam and Bengt Lindqvist

Lewis Thomas et al. (12, 13) in experiments on rabbits, observed that epinephrine or norepinephrine injected intradermally within 4 hours after intravenous injection of endotoxin from Gram-negative bacteria (E. coli, S. equi, and others) produced haemorrhage into the skin and, in many instances, necrosis of hair follicle cells at the site of injection. The lesions were fully developed after 12 to 14 hours and were produced by doses as small as 1 μg of intravenous endotoxin and 5 μg of intradermal epinephrine. Referring to this skin lesion produced by epinephrine Thomas and his collaborators (12) stated that "we have come to regard it as one of the most constant and reproducible of all the biological properties of endotoxin". Without pre-treatment with endotoxin no reaction appeared after injection of 200 μg of epinephrine once hourly for 8 hours into the same skin area.

Simultaneous injection of heparin did not prevent the appearance of the reaction, in contrast with the cutaneous reaction to epinephrine after intravenous injection of horse-serum in rabbits, described by Gatling (4).

After repeated small daily doses of endotoxin the rabbits became "tolerant" and did not react to epinephrine even when a large dose of endotoxin was given immediately before the epinephrine injection.

Neter et al. (9, 10) found that an intradermal injection of epinephrine after an intravenous injection of extract from a strain of S. aureus produced a similar skin reaction in rabbits. Neter et al. (10) observed a positive skin reaction in 75 % of the cases in rabbits after intravenous injection of 5 μg of endotoxin and intradermal injection of 0.1 mg of epinephrine.

According to earlier experiences, intravenous injection of endotoxin in rabbits produces fever, shock, leukocytosis, thrombocytopenia, petechial haemorrhages and focal necrosis in internal organs.

In man endotoxinaemia may be present in infections with Gram-negative bacteria; under certain conditions endotoxins from the intestinal flora of Gram-negative bacteria may possibly enter the circu-

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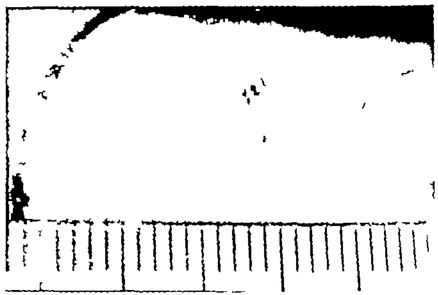


Fig 1 Local reaction to 0 1 mg of epinephrine injected intradermally

lating blood (3 5) A clinically usable method for demonstration of endotoxins in the blood is lacking however To find out whether a test with intradermal epinephrine similar to that described by Thomas (12) can be used for diagnostic purposes in man, we studied the local reaction to intradermal epinephrine in normal subjects, in patients without obvious causes for endotoxinaemia and in patients with probable causes for endotoxinaemia Most of the latter were severly ill with acute renal failure many of them being infected with Gram negative bacteria.

Technique and reaction

Using a fine needle (0 45 mm in diameter) 0.1 mg of epinephrine (0 1 ml of a 1 1 000 dilution) was *injected intradermally* as a rule on the inside of the lower leg The injection was accompanied by slight palpitation of the heart of very short duration, transient pallor of the skin at the site of injection, and thereafter one of the following alternatives was noted

1 No further reaction bleeding in the needle track occurred in some cases.

2. Local diffuse bluish discoloration appeared within a few minutes and disappeared after a few hours such reactions were designated as negative.

3 A reaction appearing after 3 to 6 hours (fig 1) characterized by a) a bluish purple colour b) even and sharp definition, and c) slight elevation of the skin the reaction reached its maximum after about 24 hours and persisted for a few days. This reaction was designated as *positive* For practical reasons we considered the reaction positive only if the sum of two diameters at right angles to each other measured at least 3 mm. For 44 observations this sum averaged 5.0 (3—9) mm.

In 6 cases the skin was excised 24 hours after the injection of epinephrine, in 4 cases post mortem. Microscopical examination showed a fresh haemorrhage and, in some cases, degeneration of connective tissue fibrils and sparse infiltration of leukocytes in the needle track.

In the first 150 subjects included in the material reported below both the aforementioned dose (0 1 mg of epinephrine) and 0 01 mg of epinephrine (0 1 ml of a 1 10 000 dilution) were injected. In only 1 case did a positive reaction occur after the lower dose the higher dose also produced a positive reaction in this case

Epinephrine reaction in normal subjects

Eighty-seven injections were given in 77 subjects, 53 men and 24 women whose ages ranged from 19 to 44 years, the mean age being 28.1 years. Four persons (5.2 %)

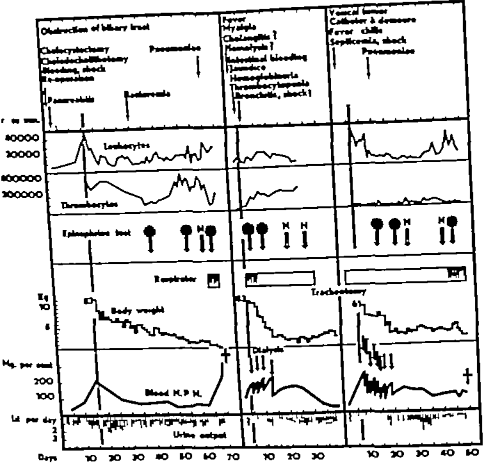


Fig. 2. Three illustrative cases. N = no reaction to epinephrine.

showed positive reaction. They were all healthy young men aged 20 to 34 years. After repeated injection few days later positive reaction occurred in two of these four men. In one of these two positive subjects gastroenteritis developed two days later. After his recovery the reaction was negative.

Epinephrine reaction in patients

Two hundred and ninety-five tests were made in 189 in-patients at this clinic, the Medical Clinic or the Rheumatological Clinic. Ninety-five were men and 94 women, and their mean age was 51.0 (15—88) years.

The heterogeneous material of patients was divided into two groups. Patients with severe renal failure and patients without severe renal failure.

Group I. Patients with severe renal failure (107 cases)

This group includes all patients with acute or chronic renal failure, who during the period of study had N. P. N. level exceeding 100 mg/100 ml or oliguria-anuria. Eighty patients were treated with the artificial kidney. The majority had tubular necrosis (65 cases) and the rest had pyelonephritis, glomerulonephritis, nephrosclerosis, postrenal obstruction, polycystic kidneys, periarteritis nodosa, etc. The mortality was high (57 %), which may largely be ascribed to the high incidence of severe basic diseases (f. i. pancreatitis, severe trauma, perforating ulcer) and of complications, such as severe infections (for instance sepsis, abscess, peritonitis, bronchopneumonia) circulatory and respiratory disorders, bleed-

Table I Cutaneous reaction to epinephrine in normal subjects and in patients

Group	Patients			Testings		
	Total no.	Positive reaction		Total no.	Positive reaction	
		No.	%		No.	%
Patients with renal failure	107	31	29	202	44	22
Patients without renal failure	82	5	6	93	6	6
Normal subjects	77	4	5	87	6	7

ings, fever intestinal paralysis, cerebral lesions, etc. The intradermal test was repeated in many of these patients during recovery after diuresis had increased and N P N had begun to fall. At one-third of the testings the patients general condition was relatively good.

Group 2 Patients without severe renal failure (89 cases)

This group includes patients with diseases such as ulcerative colitis, duodenal ulcer cancer with varying location, pernicious anaemia lupus erythematosus, bronchial asthma, adrenocortical insufficiency hypothyrosis, myocardial infarction endocarditis, subdural haemorrhage, psoriasis, rheumatic fever rheumatoid arthritis, liver cirrhosis, and/or renal disease, such as pyelonephritis with or without papillary necrosis, glomerulonephritis, myeloma, sarcoidosis, amyloidosis, malformations of the kidney hydronephrosis, renal cysts, renal damage due to trauma, renal emboli, malignant hypertension, etc. Neither oliguria anuria nor a N P N level exceeding 100 mg/100 ml was found in these patients during the period of study The renal disease predominated in 33 of the patients. The majority were ambulant and their general condition was good, though a few of them were in a poor condition. Four of these patients died in the hospital.

Group 1 differs from group 2 not only as regards renal function but also in its higher incidence of infections, higher incidence of circulatory disorders and changes in the blood picture, and in the patients poorer general condition. In our analysis of the material we have considered these differences.

Results

In 36 patients (19 %) 25 men and 11 women with a mean age of 51.3 (18—74) years, a positive reaction occurred 50 or 17 % of the 295 tests were positive. Fig 2 shows three illustrative cases.

It will be seen from table I that a positive reaction occurred in 5 % of the normal subjects and in 6 % of the patients without severe renal failure, as against 29 % in the group with severe renal failure The difference is statistically significant The conditions were probably more favourable for the development of endotoxinaemia in the last named group.

Renal function. It will be seen from table II that the percentage of positive epinephrine reactions was higher in malignant hypertension, tubular necrosis and pyelonephritis than in glomerulonephritis and other renal diseases. At a normal N P N level the epinephrine reaction was positive in 7 % in uraemia with N P N below 200 mg/100 ml it was positive in 21 % and at N P N exceeding 200 mg/100 ml in 21 % of all the testings. There was no reaction in 3 patients with N P N exceeding 300 mg/100 ml. In oliguria anuria positive reactions were recorded in 25 % of the testings.

Infection In the presence of sepsis, peritonitis, abscess, bronchopneumonia or other severe infections a positive reaction occurred in 26 % of the testings (table

II) It was not regularly positive in any type of infection, however

Gram-negative bacteria or staphylococci often grew in cultures from urine and secretions from the trachea, mouth, nose, wounds, etc., in the patients with severe renal failure. Gram-negative bacteria were present on 130 occasions in such cultures within 14 days before the day of the epinephrine test. 20 % of the tests were positive. The tests were negative in 4 cases in which, on the same day Gram-negative rods grew in blood-cultures.

Fever above 38 C was present at the time of testing in 27 % of the cases when a positive reaction occurred and in 15 % when there was no reaction.

Changes in the blood-picture etc. The epinephrine reaction was positive in 11 % of 160 tests at a leukocyte count below 11,000 and positive in 26 % of 121 tests at a leukocyte count exceeding 11 000 cells/mm³

The ESR was raised in most cases. It was below 40 mm/h t 73 tests the reaction was positive in 7 %. ESR was 40—99 mm/h t 89 tests the reaction was positive in 29 At 76 tests ESR exceeded 100 mm/h the reaction was positive in 13 %

Of 46 tests in 34 cases with a haemoglobin concentration below 7 7 g/100 ml, 22 % were positive of 145 tests in 120 cases with haemoglobin between 7 7 and 12.2 g/100 ml, 18 were positive, and of 74 tests in 62 cases with haemoglobin exceeding 12.2 g/100 ml, 14 % were positive

The reaction was positive in 40 % of 38 tests at a thrombocyte count below 80,000 cells/mm³ and in 18 % of 181 tests at higher thrombocyte counts.

Circulatory disorders Shock was common in our cases. 23 of 51 tests were positive t a systolic blood-pressure below 100 mm Hg 19 % of 31 tests were positive during treatment with vasoconstrictor agents, mostly metaradrin and sometimes nor epinephrine. 26 % of 19 tests were positive during treatment with dextran 23 % of 18 tests were positive during corticosteroid therapy for shock. No reaction occurred in 2 severely ill patients who died 6 and 10 hours later the period of observation may have been too short, however

A positive reaction was obtained in 34 % of 32 tests in 28 patients with a cold sweat, in 20 % of 65 tests in 45 patients with peripheral cyanosis and in 39 of 65 tests in 42 patients with markedly yellowish pale skin.

General condition. On clinical grounds the patients were divided into two groups according to their general condition

a) Patients with relatively good general condition who were ambulant or able to sit in a chair for at least some minutes 9 % of 164 tests were positive

b) Patients with poor general condition and shock, coma, stupor paralytic ileus, convulsions etc. on the day of the test most of the patients in this group had severe renal failure 27 of 131 tests were positive.

Thirty-seven patients in group b) were treated in a respirator One patient waking up from poisoning with soporifics was referred to group a) because his general condition was elatively good although he was in the respirator Group b) also includes all the patients who needed vasoconstrictor agents to maintain blood-pressure. Thirty-eight out of 44 somnolent patients were considered to be in a poor general condition (group b) Three patients, of whom one had a N P N level of 226 mg, the other a haemoglobin concentration of 4 7 g/100 ml, and the third a temperature of 39.2 C were referred to group a) because their

Table II Cutaneous reaction to epinephrine in patients with renal diseases

Diagnoses	Patients			Testings		
	Total no.	Positive reaction		Total no.	Positive reaction	
		No.	%		No.	%
Malignant hypertension	6	3	50	10	3	30
Tubular nephritis	65	20	31	121	31	26
Pyelonephritis	54	14	26	86	16	19
Papillary necrosis	13	3	23	13	3	23
Glomerulonephritis	22	2	9	29	3	10
Other renal disease	33	2	6	46	2	4
N P N. 0—45 mg/100 ml	89	7	8	89	6	7
46—199	118	27	23	177	38	21
200 and higher	20	5	25	28	6	21
Oliguria — anuria	77	24	31	132	33	23

Table III Cutaneous reaction to epinephrine in severe infections

Infections	Patients			Testings		
	Total no.	Positive reaction		Total no.	Positive reaction	
		No.	%		No.	%
Sepsis	13	7	54	15	7	47
Peritonitis	10	4	40	14	4	29
Abscess	16	6	37	26	6	23
Bronchopneumonia	23	7	30	30	7	23
Other severe infection (cholecystitis, acute pyelonephritis, enterocolitis)	11	3	27	18	5	28
Total no. of patients with severe infections	50	19	38	81	21	26

general condition was relatively good. In this way the border line between the two groups became vague.

Diagnosis It will be seen from the appendix (p. 471) and from tables I—III that most of the patients with a positive reaction had very severe diseases

Mortality 61 % of the patients with positive tests and 26 % of the patients with negative tests died in the hospital.

Symptoms Three-quarters of the patients with positive tests had cerebral symptoms in the form of coma stupor somnolence, confusion or excitation. More than half the patients had yellowish pale skin, a cold sweat or tachycardia (heart rate exceeding 100 beats/min.) Peripheral cyanosis fever and a systolic blood-pressure below 100 mm Hg were each noted in one-third to one-fourth of the cases. About one-eighth of the patients had symptoms of intestinal paralysis, intestinal bleeding convulsions and tachypnoea (respiration of more than 30 to the minute)

Heparin. Fifty tests were made during heparinisation in connection with dialysis treatment in severly ill patients coagu-

bine-time was markedly prolonged. 22 % of the tests were positive. No other investigations of coagulation and bleeding were made in this study

Re-testing In 139 out of 189 patients, particularly those with positive reactions, the test was made more than once in 1 patient 6 times. Eleven patients with a positive reaction early in the course of the disease were re-tested during recovery and on these occasions no reaction occurred in any of them. In 3 cases the reaction became positive after having been negative at earlier tests. In 5 cases the reaction changed from positive to negative and again to positive at repeated tests. There seems to be a parallel relationship between deterioration of the disease and the change-over from a negative to a positive test. Fig 2 shows such cases.

Comments

In 40 individuals we observed a characteristic reaction ("positive") at the site of intradermal injection of 0.1 mg of epinephrine Microscopical examination of the skin from six positive tests showed local infiltration of erythrocytes in the tissue. We consider that this reaction is analogous to the epinephrine reaction observed by Thomas et al. (15) in experiments with endotoxin-treated animals on the following grounds

1 Haemorrhage into the skin occurred after injection of epinephrine

2 The reaction appeared after 3—6 hours and reached its maximum after 24 hours

3. The reaction had the same characteristic sharp definition and the same colour as the Thomas reaction

4) Similarly to the Thomas reaction but in contradistinction to ... horse-serum, we obtained a positive reaction during heparinisation as well

5) The skin reaction to epinephrine occurred principally in infected severely disabled patients with renal failure in whom it may be reasonably presumed that conditions favouring the development of endotoxinaemia were present

6) Many of the patients with a positive skin reaction to epinephrine had fever shock, leukocytosis, thrombocytopenia, petechial haemorrhages and in 3 cases, focal necrosis in the liver and spleen. Similar reactions have been experimentally produced in animals with endotoxin. As the patient's condition improved the skin reaction became negative

7) On theoretical grounds acute renal failure has in some cases been suspected to be due to the release of endotoxin (14) We observed a positive skin reaction to epinephrine especially in patients who had developed acute renal failure a few days previously but, of course, this was in a selected material and at that particular time the patients were in a poor general condition because of uraemia, infection, and other disorders.

No epinephrine reaction occurred in some cases, despite the presence of Gram-negative bacteria in the blood. However this fact does not seem to rule out the possibility that endotoxin may give rise to a cutaneous response to epinephrine. Animal experiments have shown that administration of repeated doses of endotoxin brings about a temporary state of tolerance and that no epinephrine lesions occur in animals thus rendered "tolerant"

We wish to emphasize that the aforementioned similarities with the Thomas reaction in experimental animals can only be taken as indirect evidence in support of the hypothesis that the skin reaction to

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APPENDIX

Diagnoses in patients with a positive skin reaction to epinephrine

1. Cholecystitis, choledochoduodenostomy necrotic pancreatitis, subphrenic abscess, septicaemia, myocarditis, uraemia.

2. Cholecystitis, cholecystectomy local peritonitis, pancreatitis, tubular necrosis, uraemia.

3. Cholecystitis, cholecystectomy pancreatitis, tubular necrosis, uraemia.

4. Liver cirrhosis, acute cholangitis, explorative laparotomy chronic pancreatitis with acute exacerbation, septicaemia, tubular necrosis, uraemia.

5. Cholangitis, haemolysis, thrombocytopenia, tubular necrosis, uraemia.

6. Urethral stricture, periurethral abscess, septicaemia, toxic hepatitis, tubular necrosis, uraemia.

7. Perforated gastric ulcer gastric operation, purulent peritonitis, tubular necrosis, uraemia, bronchopneumonia.

8. Gastroenteritis, tubular necrosis, uraemia.

9. Ulcerative endocarditis, septicaemia, pneumonia, tubular necrosis, uraemia, splenic infarction.

10. Chronic polyarthritis, renal papillary necrosis, uraemia, endocarditis, septicaemia, bronchopneumonia.

11. Ruptured aortic aneurysm, abdominal operation, shock, tubular necrosis, anuria, uraemia, bronchopneumonia, intestinal perforation, peritonitis, ileostomy septicaemia.

12. Ulcerative colitis, tubular necrosis, uraemia, septicaemia, myocarditis.

13. Multiple fractures, tubular necrosis, uraemia.

14. Leg fractures, osteosynthesis, tubular necrosis, uraemia, septicaemia, cholecystitis, cholecystostomy panophthalmitis, bronchopneumonia, peritonitis.

15. Toxaemia of pregnancy liver necrosis, tubular necrosis, uraemia.

of intoxication by endotoxin A technique for determination of endotoxin in the tissue would be essential in getting direct evidence

We found that bleedings in the needle tracks were easily distinguishable from positive skin reactions to epinephrine. As regards patients with marked capillary fragility or haemorrhagic diathesis, as for instance scorbutics and haemophiliacs, we have not had the opportunity of studying such patients. We found no correlation between the size of the skin reaction and clinical observations or laboratory data. Possibly the size of the area depends upon the extent of the weal and the depth of injection.

One of us (H. F.) is continuing these studies with norepinephrine and other substances.

Summary

Lewis Thomas et al. (13) observed local haemorrhage and necrosis after injection of epinephrine intradermally in rabbits which had been pretreated by intravenous injection of endotoxin from Gram-negative bacteria. Thomas et al. (13) regard this skin reaction to epinephrine as "one of the most constant and reproducible of all the biological properties of endotoxin We have studied the skin reaction to intradermal injection of epinephrine in man. Our interest was focussed mainly on this clinic's cases of acute failure often with a surgical basic disease and infectious complications, and a clinical picture corresponding to that in endotoxin treated rabbits, i. e. fever shock, leukocytosis, thrombocytopenia, petechial haemorrhages and focal necrosis in internal organs

Using a thin needle, 0.1 mg of epinephrine was injected intradermally A sharply defined area of bluish purple discoloration of the skin at the site of injection was designated as a positive reaction if the sum of two diameters at right angles to each other was at least 3 mm. For 44 tests at which the reaction was assessed as positive this sum averaged 5.0 (3—9) mm.

The test was positive in 5.2 % of 77 normal subjects and in 6.1 % of 82 patients with various diseases. Renal disease predominated in 33 of these cases, but oliguria-anuria was not present and N P N did not exceed 100 mg/100 ml during the period of study A positive test was recorded in 29 % of 107 patients with acute or chronic renal failure and a N P N level exceeding 100 mg/100 ml. Most of these patients were treated with the artificial kidney (80 cases) and many had acute renal failure (65 cases) The conditions were probably more favourable for the development of endotoxinaemia in the 107 patients with severe renal failure than in the rest of the cases.

Among patients with severe infections, grave uraemia, leukocytosis, raised ESR, anaemia, thrombocytopenia, a poor general condition etc. the test was positive in 21 to 40 %.

The local reaction observed by us after injection of epinephrine in man is similar to that described by Thomas (12) from animal experiments. As a working hypothesis we postulate that in man, too, this reaction to epinephrine can be indicative of endotoxinaemia.

Acknowledgement

We wish to thank Professor Rune Grubb, M. D., University Institute of Bacteriology who has encouraged our interest in the clinical importance of the endotoxins.

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The Fate and Late Effects of Non-fatal Pulmonary Emboli

By

S. R. Orell

Existing information on the frequency of pulmonary embolism is based principally upon post-mortem examinations, since the clinical diagnosis is often uncertain. Small peripheral emboli give as a rule such insignificant symptoms that these are unobserved, while the symptoms of larger emboli with pulmonary infarction can in their similarity be confused with the symptoms of pneumonia or myocardial infarction.

The mean frequency of pulmonary embolism in post mortems seems to be about 10 % (7). As a rule this figure covers fresh and organizing emboli of such a magnitude that they can be considered to have affected the course of the disease. If small peripheral emboli are also reckoned in then the figure is brought up to about 25 % (4). This figure reflects the frequency of pulmonary embolism as an acute cause of death and as a complication in fatal diseases, but gives no idea of how many survive and recover from acute pulmonary embolism. If, however, the emboli leave remnants which are identifiable during the post mortem it is then possible to make a rough estimate of the number of such cases.

Small thrombi can probably be reabsorbed without remnants or leave only a moderate fibrous intimal thickening. In the larger veins of the systemic circulation, however, it is often observed that thrombi are organized into fibrous tissue which creates a cushion-like intimal thickening or often a network of strings and bands in the lumen. Such changes can be observed many years after the thrombi have developed. Similar changes in the pulmonary arteries have been described by several authors, the majority of whom regarded them as remnants after emboli (3, 10, 24, 28). But these have been sporadic cases and no consistent examinations of larger series of post mortems seem to have been made.

In recent years there has been a great deal of interest in the origin of primary pulmonary hypertension, i. e. pressure increase in the pulmonary circulation which cannot be attributed to simultaneous chronic cardiac or pulmonary diseases. The reason for the pressure increase has in a number of cases been attributed to multiple small recurring

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16 Pregnancy acute pyelonephritis, papillary necrosis, uraemia.

17 Uterine cancer (radiotherapy) urethral stricture, hydronephrosis, pyelonephritis, uraemia, pyelostomy septicaemia.

18 Vesical cancer urethral obstruction, acute pyelonephritis, septicaemia, tubular necrosis, uraemia.

19 Vesical cancer metastasis, urethral stricture, pyelonephritis, perirenal abscess, uraemia, septicaemia, bronchopneumonia, perforated duodenal ulcer

20 Chronic pyelonephritis, perinephritic abscess, nephrostomy peritonitis, paralytic ileus, tubular necrosis, septicaemia, bronchopneumonia.

21 Chronic pyelonephritis, angiography tubular necrosis, uraemia.

22 Chronic pyelonephritis, acute exacerbation, oliguria, uraemia, bronchopneumonia, septicaemia.

23 Peritoneal tuberculosis, chronic pyelonephritis, prostatic hypertrophy prostatectomy tubular necrosis, uraemia

24 Prostatic hypertrophy chronic pyelonephritis, uraemia, bronchopneumonia, septicaemia.

25 Chlorpromazine intoxication, tubular necrosis, uraemia, bronchopneumonia, septicaemia.

26. Chronic glomerulonephritis, ulcerative ileitis, pericholangitis, septicaemia, myocarditis, uraemia.

27 Subchronic glomerulonephritis, bronchopneumonia, uraemia.

28. Chronic pyelonephritis, renal papillary necrosis, malignant hypertension, uraemia.

29 Malignant hypertension, nephrosclerosis, pyelonephritis, oliguria, uraemia.

30. Malignant hypertension, uraemia.

31 Haemolysis, tubular necrosis, uraemia.

32 Acute appendicitis, abdominal fistula, hypoproteinaemia, oedema.

33. Hodgkin's disease

34 Chronic polyarthritis, auricular fibrillation.

35 Chronic polyarthritis.

36 Proctitis gravis.



b

Fig. 1. Connective tissue strings and bands in pulmonary arteries.) Broad bands at the bifurcation of the right pulmonary artery (black arrows) b) Multiple strings in peripheral lower lobe arteries (hite arrows).) Detail of b.



b

Fig 2.) 3 months old organizing embolus ith beginning recanalization. van Gieson. × 28. b) Organized embolus, 3 1/2—4 months old, with large recanalization lumina. Weigert elastic stain. × 28.) Typical string in pulmonary artery of 4th order Acute pulmonary embolism occurred 7 years before death. Weigert elastic stain. × 28.

emboli followed by obliterating vascular changes in the peripheral pulmonary arteries (10 27) This hypothesis has been supported by animal experiments (1 16) The extent to which non-fatal emboli in the after course really can give rise to pulmonary hypertension and peripheral vascular changes is not known.

The object of this investigation was to endeavour to verify earlier postulations on the connection between pulmonary embolism and connective tissue strands in the pulmonary arteries, to examine the frequency of such strands in a major series of post-mortems and to examine the connection between non fatal emboli peripheral vascular changes in the lungs and pulmonary hypertension.

Material and methods

The material consists of 1 076 adult post mortems, that is to say all the post mortems that were carried out at the Södersjukhuset during 1960 excepting cases under 30 years of age — these being chiefly foetuses and newborn children from the obstetrical and pediatrical departments. In the post mortems the thoracic viscera were removed in one piece and the pulmonary arteries were opened from the right ventricle as far out in the peripheral branches as possible, as a rule to branches of the fifth and sixth order In all cases with pulmonary emboli or remnants of these the femoral veins down as far as the fossa poplitea were examined, as were also the pelvic veins. For certain reasons the veins of the arms and the lower leg were not examined.

In no case was intravital measurement of the pulmonary artery pressure carried out. Thus the only means by which an opinion on this could be established was through measuring the thickness of the wall of th right ventricle. A mean thickness exceeding 5 mm, excepting the epicardial fat and the trabeculae, was regarded as implying pulmonary hypertension. Due to the inexactitude of this method no gradation could be established.

In several cases with organizing emboli or with macroscopically visible fibrous intimal thickening of the pulmonary arteries the affected section of the vessel was subjected to histological examination. In many such cases sections of the pulmonary parenchyma were also examined. At least three blocks from different lobes were considered necessary for a general appraisal of the small vessels and in most of the cases some 10 blocks were examined. After fixing in 5 % formalin the tissue sections were embedded in paraffin and sectioned. The sections were stained with van Gieson stain and Weigert's elastic tissue stain, in many cases also with haematoxylin-eosin and in a few cases with Ladewig's method and iron pigment stain.

Results

One hundred and ninety (17 7%) of the 1 076 post mortems revealed pulmonary embolism. This figure embraces macroscopically visible emboli of all sizes, both fresh and organizing, but it does not include cases with only connective tissue layers on the intima. A pathological-anatomical and clinical analysis of these cases was published earlier (26)

In 27 cases with organizing emboli it was possible to establish through a study of the case histories at least a general idea of the age of the emboli when death occurred In this way it was possible to make a series of histological sections of emboli of different ages varying from 72 hours up to 4 months, in which the course of the organization could be studied. In all cases the pathological-anatomical picture combined with the clinical course and the post mortem results in general, indicated that it really was a matter of emboli and not primary autochthonous thrombosis. Often the clot was laminated and in some cases there was a definite secondary autochthonous deposit on a primary embolus. Histological examination of these 27 cases gave the following picture of the course of the organization.

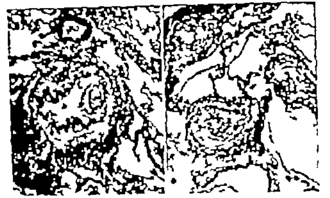


Fig. 3.) Muscular artery with small fibrinous clot covered by endothelium. van Gieson. × 100. b) Muscular artery with endothelial proliferation protruding into the lumen. van Gieson. × 100.

covered by endothelium and even subendothelially there was often a distinct elastic lamina (fig. 2c). The tissue often contained groups of small lymphocytes and in about half of the cases iron pigment was detectable. The lamina elastica interna of the vessel wall was sometimes split up though it was not generally engaged. The media was normal apart from slight sclerosis.

In 50 of the 56 cases there were changes in the femoral veins. These changes took the form of sclerotic wall thickening with obliteration of the lumen, thick fibrous ridges on the inner side of the wall or a network of transverse thin fibrous strands. These changes, which were very similar to those in the pulmonary arteries, were regarded as remnants after organized thrombi. Of the remaining cases 16 had fresh thrombi in the femoral or pelvic veins. In 10 cases there was no evidence of venous thrombosis.

An examination of the case histories of the 56 cases with connective tissue strings in the pulmonary arteries showed an age and sex division similar to that of the entire material. 75 % of the cases were between 60—70 years of age. In 13 cases it emerged from the case history that venous thrombosis and/or acute pulmonary embolism was diagnosed or suspected on some occasion from 4 months up to more than 10 years (in one case 36 years) before death. Six of the patients had been treated for heart diseases, 2 for malignant tumours, 4 for other internal diseases and 3 simply for leg vein thrombosis. In a further 25 cases the patients had undergone a major operation (17 cases) or a serious illness with a long period confined to bed, from 6 months up to more than 10 years before death. In 16 cases there was no information on previous venous thrombosis, pulmonary embolism or concomitant diseases in the histories.

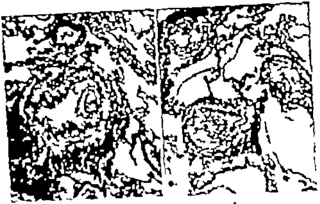
A total of 132 cases had pulmonary emboli which were macroscopically ad judged to be older emboli in different stages of organization. Twenty-five cases showed signs of pressure increase in the pulmonary circulation in the form of right ventricular hypertrophy with a mean wall thickness of more than 5 mm. In 5 of these cases the right ventricular hypertrophy was attributable to a chronic heart disease with cardiac insufficiency and chronic pulmonary congestion, and in 7 cases to a chronic lung disease such as emphysema, chronic pneumonia or pulmonary fibrosis. In 7 cases more than

Initial ingrowth of fibroblasts from the vessel wall was observed after scarcely a week and active fibroblastic proliferation after 14 days. Capillaries appeared somewhat later and were clearly visible after 14 days. Iron pigment was visible after 2—3 weeks and increased in quantity at first, but was of variable occurrence in the oldest cases. Collagen was first observed after 3—4 weeks and fine elastic fibrils after upwards of 8 weeks. The form of the elastic tissue in the organizing clot was characteristic. It appeared chiefly in diffuse form as small fine fibrils, but condensed subendothelially into distinct laminae on the surface and around the recanalization lumina. After some 4 months the clot was completely transformed into connective tissue. Naturally there were considerable variations depending upon the size of the clot and the calibre of the vessel. Successive secondary thrombotic deposits can also have delayed the organization.

The transformation into connective tissue was accompanied by a pronounced shrinking of the clot. The result of this can be a cushion like intimal thickening but if there is a strong tendency towards recanalization increasingly large lumina develop finally separated only by thin connective tissue septa. This development results in pictures which correspond entirely to the netlike strings and bridges of connective tissue on the inner wall of the pulmonary arteries which are described below (fig 2)

Connective tissue strings and bridges in the pulmonary arteries were observed in 56 of 1 076 post mortems, i e. 5 2 %. In almost half of these 56 cases there were also fresh or incompletely organized pulmonary emboli. Otherwise macroscopically no particular changes in the pulmonary arteries were observed The strings were seldom localized exclusively to one vessel section, instead they were often found multipally in arteries of different calibres and in different pulmonary lobes. Often the changes were observed in the sub-lobar pulmonary arteries, in 24 cases in 3rd order branches and in 15 cases in 4th and 5th order branches. The connective tissue strings were not uncommon, meanwhile even in the larger arteries and were thus observed in 14 cases in the lobar arteries and in 3 cases in the right of left main stem. The calibre of the strings varied from wire-fine to centimeter broad bands in the larger arteries. Often the strings were fastened at each end to the intima of the vessel with a free centre section in the lumen. This centre section, however could also be attached to the vessel wall. The strings usually ran in the longitudinal direction of the vessel although they were sometimes at right angles to it and branched in a star shaped form. Sometimes there was only one or a few strings in a vessel section but often there was a whole net work of strings in sections of up to a few centimeters (fig 1) The changes were seldom accompanied by any great degree of luminal narrowing although in one case there was a total obliteration of the left upper lobe artery

Histological examination revealed that the string like formations consisted of loose connective tissue as a continuation of the often fibrously thickened intima. There was often a centre of collagenous tissue surrounded by layers of more cell rich loose tissue. These layers were particularly clearly marked in the elastin-stained sections. The quantity of the elastin was to a great extent proportional to the collagenous content and the different layers were often separated by distinct elastic laminae. The surface was



b

Fig. 3. a) Muscular artery with small fibrinous clot covered by endothelium. van Gieson. × 100. b) Muscular artery with endothelial proliferation protruding into the lumen. van Gieson. × 100.

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Table I Pulmonary hypertension and microscopical clots in 60 cases of organizing pulmonary emboli

	Right ventr <5 mm	Right ventr > 5 mm	
		With chron. cardio-pulm. disease	Without demonstrable etiology
Microsc. clots in musc. arteries and arterioli	23	10	6
No microsc. clots in peripheral arteries	20	1	0
Total	43	11	6

half of the main branches of the pulmonary arteries were occluded. This occlusion was probably due to autochthonous thrombotic deposits on medium sized pulmonary emboli. The pressure increase in such cases has a simple mechanical explanation. In the remaining 6 cases there was no simple anatomical explanation for the right ventricular hypertrophy.

In 60 of the cases with organizing emboli or connective tissue strings or both the small peripheral pulmonary vessels were examined in sections from different parts of the lungs. Seventeen of these had right ventricular hypertrophy for which in 6 cases there was no manifest anatomical explanation. Microscopically it was often possible to observe minute clots as far out as the small muscular pulmonary arteries and sometimes even in the arterioli (fig. 3a). These consisted mainly of fibrin and it was impossible definitely to establish whether these were embolic or locally developed. Small peripheral clots of this type were found in 39 cases including all, except one, of the cases with right ventricular hypertrophy (table I).

In most of the cases the muscular pulmonary arteries revealed more or less pronounced intimal thickening. This was sometimes symmetrically concentric and sometimes limited to one sector of the cross-section, either half moon shaped or protruding into the lumen. The intimal thickening consisted of a loose or fibrous connective tissue, rich in fine elastic fibrils with a tendency towards concentricism around the lumen (fig. 4a). The degree of fibroelastic intimal thickening was described as nil, moderate or pronounced. This was based upon the degree of intimal thickness of the individual vessel and on the number of changed vessels in the sections. No consideration

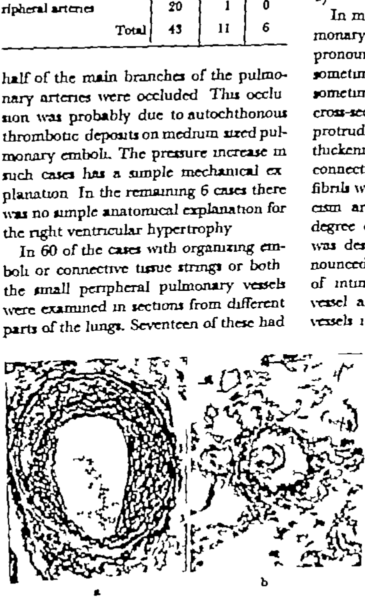


Fig. 4. a) Muscular artery with pronounced fibroelastic intimal thickening. Outer diameter 0.22 mm. Weigert's elastic stain. × 350. b) Arteriolus with pronounced fibrous thickening of the wall. Outer diameter 89 μ, inner diameter 31 μ. Weigert elastic stain. × 350.

Table II. Fibroelastic intimal thickening in muscular pulmonary arteries in 60 cases of organizing pulmonary emboli. Relation to microscopical clots in the same vessel area and to right ventricular hypertrophy

Fibroelastic intimal thickening in muscular pulm. art.	Microscopical clots not found				Microscopical clots found			
	Right ventr < 5 mm		Right ventr > 5 mm		Right ventr < 5 mm		Right ventr. > 5 mm	
	No.	%	No.	%	No.	%	No.	%
Nil	4	20	1	—	0	0	0	0
Moderate	15	75	—	—	18	80	12	75
Pronounced	1	5	—	—	5	20	4	25
Total	20		1		23		16	

Table III. Arteriolar wall thickening in 60 cases of organizing pulmonary emboli. Relation to microscopical clots and right ventricular hypertrophy

Arteriolar wall thickening	Microscopical clots not found				Microscopical clots found			
	Right ventr < 5 mm		Right ventr. > 5 mm		Right ventr < 5 mm		Right ventr. > 5 mm	
	No.	%	No.	%	No.	%	No.	%
Nil or insignificant	16	80	—	—	9	40	1	5
Moderate	4	20	1	—	14	60	10	60
Pronounced	0	0	—	—	0	0	5	35
Total	20		1		23		16	

was paid to the uncharacteristic concentric fibrous or hyaline intimal thickening of the usual arteriosclerotic type without elastic fibrils, nor to changes which were evidently the result of organizing thrombi. The degree of fibroelastic intimal thickening in the muscular pulmonary arteries in relation to the presence of minute clots in the same vessel area and to right ventricular hypertrophy is shown in table II.

In the majority of cases the arterioli showed more or less pronounced thickening of the walls. This was described as nil or insignificant if the vessel wall consisted only of an elastic lamina besides the endothelium, possibly with the inclusion of thin hyaline layer between the lamina and the endothelium. Fibrous wall thickening resulting in a certain reduction of the lumen was described as moderate. If the lumen was reduced to less than half of the vessel's outer diameter the wall thickening was described as pronounced (fig 4b) Sometimes an increase of elastin was also observed. Table III shows the arteriolar changes with the presence of minute peripheral clots and of right ventricular hypertrophy

The media of the muscular pulmonary arteries is usually of varying thickness and this is affected by such uncontrolled factors as tissue shrinkage during fixation. It is therefore difficult to establish a definite limit between normal thickness and hypertrophy The medial thickness estimates which are used as a basis for the figures in table IV are only approximate.

Table IV Medial hypertrophy and endothelial proliferation in muscular pulmonary arteries in 60 cases of organizing pulmonary emboli. Relation to microscopical clots and right ventricular hypertrophy

	Microscopical clots not found				Microscopical clots found			
	Right ventr < 5 mm		Right ventr > 5 mm		Right ventr < 5 mm		Right ventr > 5 mm	
	No.	%	No.	%	No.	%	No.	
Medial hypertrophy	4/20	20	1/1	–	5/23	20	7/16	40
Endothelial proliferation	6/20	30	0/1	–	12/23	50	11/16	70

In many cases evident endothelial proliferation was observed in the muscular pulmonary arteries without manifest relation to clots. Endothelial proliferation was sometimes so pronounced that it was almost of a papillomatous character (fig 3b) The presence of endothelial proliferation is also reported in table IV Acute arteritis in the pulmonary vessels was not observed in any of the cases, uncharacteristic inflammation of the vessel wall only occasionally and only then in connection with infarction or organizing emboli

Discussion

Connective tissue strings in the pulmonary arteries have been described by several authors and have generally been regarded as remnants of organized pulmonary emboli (3 10 24 28 29) The course of the organization has been studied in detail chiefly by Möller (25) The present investigations confirm these earlier observations. Histological examination of pulmonary emboli in different stages of organization the ages of which could be fairly well determined clinically shows that the process of organization takes about 4 months. The clot has then become completely transformed into connective tissue The volume of this decreases successively through recanalization and shrinkage and in due course there remains only a limited intimal thickening or characteristic strings and bands of connective tissue. This is fairly rich in elastin with characteristic arrangement and often contains traces of iron pigment. The strings may remain for a long time. In one case the acute pulmonary embolism seems to have occurred 36 years before death.

It can, of course, be questioned whether or not these string like formations might have developed through the organization of autochthonous thrombi Several points, however indicate an embolic origin. Thus in none of the cases which were examined histologically was there any evidence of atheromatous lesions, arteritic changes, aneurysm or malformations in the pulmonary arteries in the region of the strings. Only in 2 cases was there a chronic pulmonary disease which could have encouraged autochthonous thrombosis in one case tuberculosis, in the other sarcoidosis. The fact that in many cases strings were found in different pulmonary lobes and often simultaneously with fresher typical emboli also indicates embolic origin. Femoral vein thrombosis was found in most cases, often completely transformed into connective tissue with formations similar to those in the pulmonary arteries. In 15 cases there was a history of acute pulmonary embolism or venous thrombosis. Finally slowly de-

veloping autochthonous thrombosis in the pulmonary arteries results in a somewhat different histological picture (26)

Visible remnants after "healed" pulmonary emboli were observed in 56 (5.2 %) of the 1,076 adult post mortems. It is, of course, not possible to draw any definite conclusions on the general frequency of non-fatal pulmonary emboli from this figure. On one hand in this material the cardiovascular diseases and malignant tumours are over-represented. Such diseases have a high frequency of thromboembolic complications. On the other hand it is probable that a number of pulmonary emboli are completely re absorbed and leave no recognizable remnants. It is obvious, however that pulmonary embolism is a common occur rence even in patients who recover and that it may also appear in people who are otherwise well.

As early as 1928 (23) and 1930 (10) it was suggested that a number of cases of so-called primary pulmonary hyper tension could derive from recurrent small pulmonary emboli which, through organization, had led to obliteration of the pulmonary vascular bed. Many cases have been described in which such an origin is considered probable (5 28) In animal experiments it has also been possible to bring about a pressure increase in the pulmonary circulation through repeated intravenous injections of fragmented clot or other particles. This was followed by certain changes in the small arteries without any evident relation to the injected particles. These changes are described as endarteritis obliterans with endothelial proliferation, fibrous or often fibroelastic intimal thickening, medial hypertrophy and arteritis (1 2, 14 16 17 24) Similar vascular lesions in the human have been described, chiefly in connection with pulmonary hypertension. The connection between pulmonary embolism, peripheral vascular changes and pulmonary hypertension has not clearly been established, however

It is not fully known how often and under which conditions non-fatal pulmonary embolism in the human gives rise to hypertension. In the investigation of 57 patients with clinically diagnosed pulmonary embolism Dunér et al. found no evidence at all of chronic cor pulmonale or other serious sequela (8)

Of the 132 cases of organizing pulmonary emboli reported here 13 cases showed signs of increased pulmonary arterial pressure in the form of right ventricular hypertrophy which could not be attributed to chronic heart or lung disease. These cases are of two different types. In 7 cases more than half of the main branches of the pulmonary artery were occluded. The occlusion was proba bly the result of medium-sized emboli with the addition of secondary thrombosis. The excessive reduction of the pulmonary arterial bed is sufficient ex planation for the pressure increase (9 15 18, 20) In the emaining 6 cases there were only small, peripheral emboli with limited distribution. In these cases the pressure increase cannot be attributed to a direct mechanical effect of pulmonary emboli. Microscopic investigation of the smaller pulmonary arteries is therefore of major interest.

A systematic histological examination of the peripheral pulmonary arteries was mad in 60 of the above 132 cases. In 39 cases minute clots were discovered in the small muscular arteries and sometimes even in the arterioli. Sixteen of these cases had right ventricular hypertrophy as against 1 out of the 21 cases without such peripheral clots (table I) These observa-

tions indicate that a spreading of minute clots to the smallest arteries stimulates the development of pulmonary hypertension. However the cases where right ventricular hypertrophy was probably secondary to a chronic heart or lung disease did not really differ in this respect from the cases without such an origin. Thus the possibility of the opposite conditions cannot be excluded i. e. that pressure increase results in peripheral vascular changes with secondary thrombosis.

Fibroelastic intimal thickening in the muscular pulmonary arteries is a characteristic change which is not directly related to arteriosclerosis. As can be seen in table II this type of intimal thickening correlates with the presence of minute clots in the corresponding part of the vascular bed but it is not more pronounced in cases with right ventricular hypertrophy. Probably there exists a close connection between thromboembolism and fibro-elastic intimal thickening. An eccentric thickening is probably the direct result of the organization of a clot, whereas concentric lesions have been attributed to ischaemia in the vessel wall on account of thrombotic occlusion or of vasospasm (1 2). Many cases of chronic pulmonary congestion reveal fibroelastic intimal thickening even though emboli may not be macroscopically visible. In many such cases, however microscopic examination reveals small clots in the peripheral arteries. Ischaemia in the vessel walls due to the chronic congestion may also explain the presence of such vascular changes.

The arterioli often showed a more or less pronounced wall thickening. This was chiefly of a hyaline or fibrous nature, but an increase of elastin was sometimes also visible. The wall thickening was more pronounced in cases with minute clots in the smallest arterial branches than it was in the others. It was also particularly pronounced in the cases with right ventricular hypertrophy (table III). Arteriolar changes are thus not unusual in uncomplicated pulmonary embolism, but they show a definite increase with the development of hypertension.

Medial hypertrophy of the muscular pulmonary arteries is often observed in cases of pulmonary hypertension. In these investigations the appraisals of the thickness of the media were only approximate and thus no definite conclusions can be drawn from the results. There is, however an obvious tendency to correlation with pulmonary hypertension. Endothelial cell proliferation in the muscular arteries which has been observed in experimental pulmonary embolism was found in half of the cases that were subjected to histological examination. This change was found more often in cases with right ventricular hypertrophy and would thus seem to be stimulated by pressure increase (table IV).

The results of the investigations give no definite answer to the question of how small pulmonary emboli can give rise to pulmonary hypertension. As has already been pointed out the emboli in themselves did not cause such an extensive obliteration of the vascular bed that the pressure increase can be attributed to this alone at least in the 6 cases in this material. Fibro-elastic intimal thickening in the muscular pulmonary arteries is equally pronounced in pulmonary embolism, irrespective of whether right ventricular hypertrophy exists or not. On the other hand in cases of right ventricular hypertrophy more pronounced obliterating arteriolar changes are often observed. This may be compared with certain hypertonic conditions in the systemic circulation. In

phaeochromocytoma the release of nor adrenaline gives rise to intermittent pressure increase. By degrees this leads to hyaline thickening of the arterioli in different organs, chiefly the kidneys. When these vascular changes have developed to a certain degree the pressure remains constantly elevated. There is a similar mechanism behind the Goldblatt effect (12, 13). In the case of recurrent pulmonary embolism it is possible that by mechanical or humoral means the emboli may give rise to spasm in the peripheral pulmonary arteries with resultant intermittent pressure increase. By degrees this results in obliterating arteriolar changes leading to constant and increasing hypertension. Embolic occlusion and fibro-elastic intimal thickening undoubtedly contribute to this development. This hypothesis is supported by the observation that a crop of minute clots in the extreme pulmonary arteries almost always occurs in those cases which develop pulmonary hypertension. Barnard has reached similar conclusion on the basis of his experimental investigations (1, 2).

Summary and conclusions

The organization of non-fatal pulmonary emboli and the subsequent conditions has been studied in 1,076 consecutive adult post mortems in the Söder sjukhuset during 1960. The results of the investigations are summarized as follows

1 The organization of a medium-sized embolus is completed and the clot transformed entirely into connective tissue after some four months.

2. Through recanalization and shrinkage the organized clot is gradually reduced to thin strings or bands of connective tissue which remain for a long period.

3 5.2 % (56 cases) of the post mortems revealed such connective tissue strings.

4 10 % of the 132 cases of emboli in different stages of organization showed signs of pulmonary arterial pressure increase which could not be attributed to chronic heart or lung disease. In half of these cases there was massive occlusion of the main pulmonary arteries. In the other half there were only small emboli which in themselves could scarcely have led to any significant reduction of the arterial bed

5 Microscopic investigation of 60 cases with organizing emboli often revealed minute clots in the muscular pulmonary arteries and sometimes even in the arterioli. These clots were observed in practically all those cases which also showed signs of pulmonary hypertension

6. Fibro-elastic intimal thickening in the muscular pulmonary arteries is a characteristic change which seems to accompany pulmonary embolism. This change was not more pronounced in the cases with right ventricular hypertrophy than in the others.

7 Arteriolar wall thickening and obliteration was clearly more pronounced in cases with right ventricular hypertrophy The same is probably true of medial hypertrophy in the muscular arteries.

8. The consequent hypothesis on the pathogenesis of pulmonary hypertension in recurrent pulmonary embolism is based on the above observations and also upon comparison with hypertonic conditions in the systemic circulation. By mechanical or humoral means small recurring emboli give rise to spasm in the peripheral arteries resulting in intermittent pressure increase By degrees obliterating changes develop in the small vessels, chiefly in the arterioli, leading to a constant hypertension.

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From the First Medical Department and the Central Laboratory Sahlgren's Hospital, University of Gothenburg Sweden

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For rational approach penetrating bilateral functional studies seem to be necessary to decide the type or extent of operation or to balance the choice between active surgery or active medicamental treatment. These cases, however also offer an opportunity to study factors of general interest in renal physiology and in particular the influence of a varied filtration pressure on the excretion of electrolytes. The studies of Mueller et al. (18) as well as those of Blake et al. (3) on the effect on the excretion of Na and H_2O of mild, severe or total clamping of one main renal artery or one of its major branches in the dog have been suggestive. The penetrating work by Davidson et al. (7) has thrown light on the relationship between filtration and the excretion of Na^+ and K in unilateral clamping in the dog.

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Clinical material

The extensive use of lumbar aortography in the investigation of cases of hypertensive disease has led to the finding of fairly large

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Addendum

Since this manuscript was completed another paper has appeared on connective tissue strings in the pulmonary arteries (KORN, D GORE, I., BLENKE, A. & COLLINS, D P: Pulmonary arterial bands and webs: An unrecognized manifestation of organized pulmonary emboli. Amer J Path. *40*: 129 1962) This work comprises a study of 32 cases.

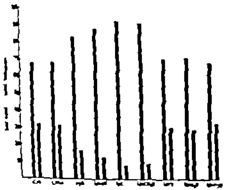


Fig. 1. Bilateral renal function in main stem unilateral renal artery stenosis. Function expressed as percentage of total basal function which has been set at 100 %. Right columns = stenosed side. Average of 7 cases.

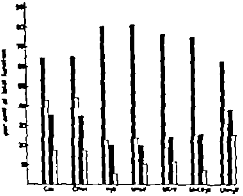


Fig. 2. Effect of acute blood pressure reduction in main unilateral renal artery stenosis. Right pair of columns — stenosed side.
■ Basal condition
□ Acute reduction of B.P. (i.e. hexamethonium)

the elected blood pressure level was obtained, the pressure was kept constant during the rest of the clearance study. The time period necessary for bringing the blood pressure down to that level was 10—20 min. In order to study more closely the changes brought about during the phase of pressure fall, the urine was in some cases collected in 5 minutes samples.

Hydration

In another series of patients, particularly those where initial blood pressure levels were not high enough to bring about decisive changes with hexamethonium, we performed intense hydration. This was done by means of special pump for rapid intravenous delivery and with a maximum delivering capacity of 30 ml/min. We brought up the infusion rate by steps and watched for the response, the final infusion rate being to some extent dependent upon the response. Usually we could obtain rates of about 20 ml/min. A history of earlier heart decompensation was considered as contraindication against higher infusion rates.

Chemical methods

Analysis of inulin was made according to the colorimetric method of Hubbard and Loomis (13). P-amino hippuric acid was analyzed with Brun's method (5). Na and K were determined with a flame spectrophotometer with lithium internal standard. Cl was titrated with mercurinitrate by Brun's (6) modification of Schales and Schales (19) method. Total-CO_2 was measured according to the method of Lehmann (15), using Conway' diffusion unit. phosphorus according to a slight modification of the method of Fiske and Subbarow (9) and NH with Kjeldahl destillation technique. Osmolarity of the urine was determined in an osmometer using a thermistor-bridge. pH was measured electrometrically on a radiometer PHM 22. Titrable acidity of urine was measured with Na hydroxide according to a modification of the method of Folin (16).

Results

The results obtained in a series of clearcut main unilateral renal artery stenosis are first described. This has been done in an attempt to establish if there occurs a definite pattern in this condition which may be considered the simplest prototype of renovascular disease.

Fig. 1 shows the average results in 7 cases of main unilateral renal artery stenosis with more than 10 % side dif

number of renal artery lesions of varying kinds, localization and degree, main stem lesions, branch lesions polar lesions, renal artery aneurysms as well as renal artery stenotic lesions coexisting with parenchymatous disease of the kidney of various kinds (chronic pyelonephritis, polycystic kidney and chronic glomerulonephritis) Renal aortography will often reveal minor lesions of questionable hemodynamic importance, usually atheromata. Bilateral function studies (a rather formidable diagnostic procedure) were omitted in a number of cases, where the clinical condition from the outset eliminated thoughts of surgery either because of the state of the aorta, the coronary or cerebral arteries or because of moderate blood pressure levels. In a few cases, where the lesion from renal aortography seemed insignificant but the pressure was high, bilateral function studies have been performed. The clinical condition, the blood pressure level as well as the varying success in getting the bilateral study under way smoothly and without technical difficulties decided whether to perform a short study with a few periods or a prolonged study with up to 20 clearance periods. In the latter case experimental changes were performed either in the form of acute reduction of blood pressure, intense hydration or in a few cases by infusing vasoconstrictor agents. In the present study we have summarized our results in 56 experiments in 54 patients with renal artery lesions. For purposes of comparison we also included 5 cases of chronic, mainly unilateral, pyelonephritis.

Methods

The patients were investigated in the morning in a fasting and well hydrated state. This was maintained by frequent drinking during the whole procedure or by an intravenous infusion of 3.5 % glucose solution. In cases where hexamethonium infusion was used the patients were not allowed to drink after the infusion had been started, due to the increased risk of vomiting. In those cases, where the aim was to investigate ion excretion during low and high urinary output, the patient was only allowed to drink 500 ml water before the examination. If possible all active antihypertensive agents were omitted at least 48 hours before the test. In any case we always excluded electrolyte-active antihypertensive drugs. These were usually omitted several weeks before the clearance procedure.

After premedication with hydromorphone-atropine and topical anesthesia of the urethra with xylocaine gel, cystoscopy was performed. Ureteral catheters (Charrière 6—7) were inserted into the ureters. A soft rubber catheter was placed in the bladder to detect leakage around the ureteral catheters. If leakage appeared, the side was detected by injecting the catheters with indigo-carmine before and after the actual measurements. This checking for leakage was also performed if, for instance, because of technical difficulties or pathological changes at the ureteral ostium, it was impossible to put the catheter into one of the ureters. The urine from that side was then collected through the bladder catheter. The urine was watched for signs of infection for some days after catheterization and as a routine antibiotics were administered after the test.

Frequent sphygmomanometric B.P measurements were made throughout the procedure. Rectal temperature was controlled immediately before and after the test.

After the injection of suitable priming doses of inulin and PAH a sustaining infusion of these substances in glucose or physiological saline solution was administered by means of a motor driven syringe at a slow constant rate. After an equilibration time of 30—40 min. actual period measurements were started. Appropriately timed arterial blood samples (indwelling arterial catheter) were collected under liquid paraffin. The lengths of the urine collecting periods were varied entirely according to the level of the urinary output. The urine volume might be extremely low in the presence of severe arterial stenosis, especially after lowering the blood pressure, and in such instances the collection periods had to be increased from the usual length of 10—20 min. to 60—90 min.

Blood pressure reduction

Acute blood pressure reduction was brought about by an intravenous drip of hexamethonium in 5.5 % glucose. The amount of hexamethonium needed, in order to bring about a blood pressure reduction as close to normal as suitable varied between 20 and 100 mg. After

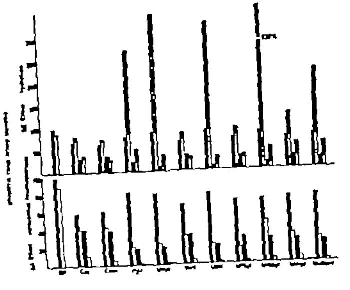


Fig. 4. The effect of intense hydration and of acute reduction of blood pressure in two typical cases of main unilateral artery stenosis.
■ Basal condition
□ I. hexamethonium
□ Hydration

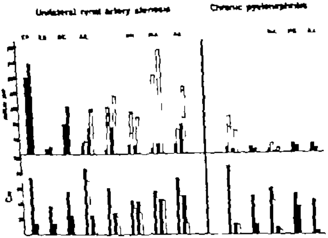


Fig. 5. Inulin clearance and concentration index (Inulin U/P) in main unilateral renal artery stenosis contrasted with the conditions in asymmetric chronic pyelonephritis. The effect of acute reduction of blood pressure.
■ Basal condition
□ I. hexamethonium

both sides, particularly on the stenosed one, on rapid lowering of blood pressure (white columns). In the series of chronic asymmetric pyelonephritis, where the clearance is performed under the same conditions of hydration, the inulin U/P ratio is lower as might be expected, and the side with the smaller filtration will show the lower inulin U/P-ratio which might also be expected. In the 2 studies of cases of pyelonephritis where blood pressure reduction was performed, the decrease in inulin clearance on the most affected side seemed less than that occurring in unilateral renal artery stenosis and the increase of the inulin U/P ratio was slight.

In contrast to the above results fig. 6 illustrates the pattern obtained when a major branch of one renal artery is completely occluded. This was a male of 53 years with auricular fibrillation who

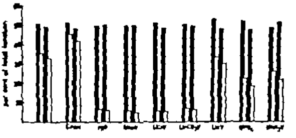


Fig 3. Main stem unilateral renal artery stenosis with no recognizable side difference in filtration. Slight but inconclusive lowering of the output on the stenotic side (right columns) during acute blood pressure reduction.

■ Basal condition

□ Acute reduction of B.P (I.v hexamethonium)

ference in filtration. Only technically fully successful experiments have been included. It may be seen, that the reduction in the excretion of K^+ PO_4[1] and NH_4 in the stenotic kidney is of the same order and also closely parallels the reduction in C_I and C_{PAH} while stenosis produces a much more marked and closely parallel reduction in H_2O Na^+ Cl^- and total CO_2

Fig 2 demonstrates the effect of acute blood pressure reduction in 4 cases of main stem unilateral artery stenosis. C_{In} and C_{PAH} were approximately halved during reduction of B P on the stenotic side, while the decrease in these parameters is less on the non-stenosed side The excretion pattern of H O Na Cl+ and total CO_2 is changed in a parallel manner As the drop on the non stenosed side is very severe, acute blood pressure reduction does not seem to contribute in this respect to further elucidation. The reduction in the output of NH_4 seems less.

Fig 3 shows averaged values for 2 cases of clearcut radiological stenosis with no side difference in filtration. The pattern of excretion of ions during the basal condition as well as during blood pressure reduction only showed very slight side difference. Considering the errors which may be encountered in this complex type of experiment, conclusions regarding the effect in renal function cannot be drawn.

Fig 4 shows the very definite changes occurring on a very moderate blood pressure reduction in the lower part of the curve and also the increased side difference particularly occurring in H_2O Na^+ Cl^- and total CO_2, where the excretion is markedly lowered on the affected side. In the upper part of the curve there is seen the effect of acute intense hydration. B P is somewhat lowered On the nonstenotic side there is some increase in filtration and plasma flow a marked increase in the output of Na^+ H_2O Cl^- and particularly total CO_2, while the increase of K^+ and PO and NH_4 is small. On the stenosed side the increase in H_2O Na^+ Cl^- and total CO_2 and NH_4 is small and in the case of K^+ and PO_4 there seems to be no change Thus during forced diuresis the stenosed kidney fails to respond or responds very little. Also the parallel behaviour between K PO4 and NH is stressed on both sides as well as within the 'group H_2O Na Cl and total CO_2. In particular the side difference of total CO_2 excretion between stenosed and non stenosed side seems striking

Fig 5 points out the characteristic differences between unilateral renal artery stenosis and chronic asymmetric pyelonephritis in a number of patients. It is seen, that on the side where the filtration is lower as a result of unilateral renal artery stenosis, the concentration index (inulin U/P-ratio) is always higher Also this higher U/P ratio is markedly increased on

[1] PO has in the following been used both for plasma and urine regardless of the actual concentrations of $H_2PO_4^-$ or HPO_4^{--}

nosis the side difference in the output of Na was generally larger than the side difference in filtration, while the opposite was true in chronic pyelonephritis. A case of severe bilateral renal artery stenosis with a very large side difference (upper right corner) falling exactly on the line denoting a perfect symmetrical reduction of the output of Na in relation to the reduction of filtration seems very provocative of thought. This case excreted exactly 5 % of the calculated filtered Na on both sides, the inulin clearance being 0.75 ml and 15 ml/min. respectively

Postoperative analyses in partial therapeutic failures have been performed only in a few cases. Some interesting observations have been made and will be reported elsewhere. In a study performed 3 months after operation we have observed a formerly Na -retaining and well-concentrating kidney change into a Na losing and less concentrating state as compared with the contralateral unoperated kidney This finding seems to correspond to the observation made by Johns Hopkins group of a copious output of Na during the postoperative period. We have interpreted this as tubular damage (probably transitory) caused at operation, most probably due to the clamping

Bilateral renal artery stenosis

In table I data on a number of cases with bilateral renal artery stenosis have been given. It is seen that when function is closely the same as regards filtration the excretion of electrolytes does not give any consistent pattern. Osmolarity as well as inulin U/P-ratios do not always produce the highest figures on the side with a lower inulin clearance. In other words, the help obtained from data such as these is only in the way of a gross estimation of the degree of kidney damage on both sides.

Branch lesions

In only 3 cases of our total 90 cases of arteriographically proven renal artery lesions was there unequivocal evidence of a branch lesion. In 2 of these the side difference in inulin clearance was more than 10 %, and there was a faint suggestion of the same pattern in regard to the ions as in the main stem lesion cases.

Co-existence of renal artery lesions and primary parenchymatous disease

Of the 7 cases, where this combination was diagnosed a 63-year-old female had chronic papillonecrotic pyelonephritis with sudden development of malignant hypertension and uremia. The diagnosis was verified by the autopsy findings. No functional study had been performed. A 33-year-old male with history and biopsy findings of chronic glomerulonephritis exhibited marked hyperlipemia and severe bilateral, atherosclerotic renal artery changes. Repeated attempts of ureteral catheterization were unsuccessful. In a 67 year-old female with xanthomatosis, phenacetin abuse, interstitial nephropathy and moderate bilateral stenosis we thought it inadvisable to continue our studies. Of the remaining 4 cases number 2 in table II had a history of a few attacks of left-sided acute pyelonephritis and moderate signs of chronic pyelonephritis on needle biopsy There were no signs of actual activity at the time of investigation. The left kidney contributed about 1/3 of the total filtration. The inulin U/P-ratio and osmolarity were higher on the left side. The preoperative plan was to save the kidney if there was a stenosis with a definite gradient, and if the kidney sur

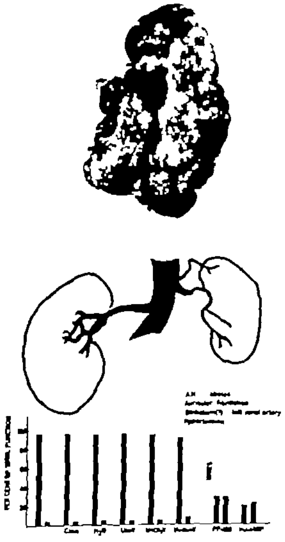


Fig 6. The effect of complete occlusion of a major branch of one renal artery (embolism)

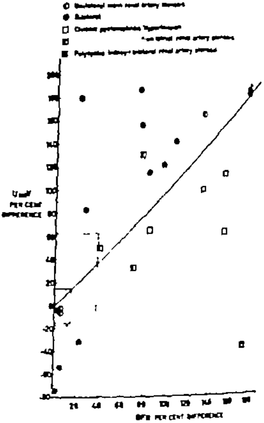


Fig 7 Side difference in sodium output versus side difference in filtration. Cases of main unilateral renal artery stenosis, bilateral renal artery stenosis, chronic pyelonephritis and chronic pyelonephritis combined with renal artery stenosis. One case of polycystic kidney with bilateral renal artery stenosis. Plotted against the background of the data of Baldwin et al. (1 14) Points above the bisecting line denote that the sodium output has fallen disproportionately more than filtration. Below the bisecting line vice versa. Inner and outer rectangles contain normals and essential hypertensives respectively (Baldwin's material).

had experienced an acute flank pain 2 months before the study Arteriographically a major branch of the left renal artery seemed to be completely occluded The volume of urine from the left kidney was only 4 % of the total output but the quality of the urine was in all respects equal to that of the healthy side. This was interpreted as caused by a small part of the kidney filtering under entirely the same filtration pressure as the contralateral kidney It was also thought that the rest of the kidney was completely non excretory The operation showed a yellow shrunken kidney with some islands of apparently normal tissue, the situation of these islands being in good agreement with those branches of the renal artery which seemed to be patent arteriographically

Fig 7 shows the data of our technically successful experiments plotted against the background of the data given by Baldwin et al. (1) In unilateral main stem ste-

Excretion of electrolytes												Conc. of urine			
$U_{Na}V$		$U_{Cl}V$		$U_{HCO_3}V$		U_KV		$U_{PO}V$		$U_{NH}V$		mOsm/kg H_2O		Inulin U/P	
R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
95	5	—	—	—	—	99	1	99	1	—	—	374	1 053	13	22
53	47	51	49	—	—	—	—	—	—	—	—	628	618	53	58
73	27	72	28	79	21	64	36	60	40	57	43	623	698	73	133
30	70	26	74	45	55	39	61	48	52	58	42	326	224	49	26
49	51	49	51	50	50	50	50	46	54	48	52	710	762	211	215
37	63	32	68	28	72	52	48	50	50	57	43	806	560	197	144
48	52	49	51	44	56	50	50	48	52	53	47	430	424	58	32

Table II. Co-existence of renal artery lesions and primary parenchymatous disease

Pat.	Age Sex	Diagnosis	Urinary output ml/min		C_{In}		C_{PAH}		Inulin U/P-ratio		mOsm/kg H O	
			R	L	R	L	R	L	R	L	R	L
M. S.	44 ♀	Phenacetin abuse + interstitial nephropathy + bil. ren. art. stenosis	0.6	0.3	41	20	153	72	66	56	5.1	469
K. K.	38 ♀	Chron. pyelonephritis + left renal art. stenosis	1.2	0.7	61	17	272	103	17	34	178	210
R. J	55 ♂	Polycystic kidneys + bil. ren. art. stenoses	0.9	0.7	20	2	89	7	35	5	468	280
A. J.	48 ♀	Chron. pyelonephritis + left renal art. stenoses	2.2	0.4	44	5	179	22	22	14	209	162

of measuring function to evaluate the importance of lesions and help with intelligent planning of surgery. Most papers agree, that the typical pattern of a unilateral main stem lesion is a kidney which excretes a smaller volume of highly concentrated urine. The reduction of the output of H_2O and Na has also been found to be disproportionately great in vascular obstruction.

A number of other problems might be discussed. Slight unilateral clamping in the dog may produce a definite decrease in the output of Na and H_2O on the clamped side, even if the filtration has not changed measurably (18). We have in several instances seen the clinical counterpart of this phenomenon. The basic functional pattern of the stenosed kidney is a marked lowering of the excretion rates of H_2O Na Cl and total CO of about the same degree. Filtration, plasma flow and excretory rates of K PO and NH_4 were less re-

Table I Bilateral renal artery stenosis Functional data — percentage distribution of total function

Pat.	Age	Sex	Urinary output		Clearances							
			% of total		C_{In} % of total		C_{PAH}		C_{In} ml/min		C_{PAH}	
			R	L	R	L	R	L	R	L	R	L
S. O	60	♀	97	3	95	5	95	5	15	1	76	4
E. K.	57	♀	50	50	48	52	47	53	38	41	170	190
E. N	63	♂	70	30	56	44	56	44	63	49	261	215
S. W	48	♂	49	51	58	42	54	46	40	29	171	144
L. L	36	♂	51	49	50	50	50	50	73	73	311	306
Ö. H.	57	♂	44	56	51	49	53	47	46	44	173	158
E. S.	59	♀	49	51	52	48	51	49	31	28	115	109

face looked moderately sound. The findings at the operation supported the preoperative plan. Thromboendarterectomy and patch graft was performed. This case has been followed for about 9 months and has remained near normotension.

In case number 3 the low inulin U/P ratio some suggestive signs in the urinary sediment and osmolarity on the side with an inulin clearance of only 2 ml/min. as well as suspicious signs in the urinary sediment made us suspect the existence of serious parenchymatous disease. However the condition was desperate with increasing azotemia. The bilateral stenosis looked severe arteriographically. Operation was undertaken as a desperate measure. Bilateral polycystic kidneys were found at operation. Thrombosis of the operated left renal artery occurred and the patient died in uremia. Retrospective scanning of the urographic and arteriographic films showed no clearcut evidence of polycystic kidney.

Incomplete and partially incomplete tests

Ten of 54 tests were more or less unsuccessful. In 4 cases the reason for noninclusion was leakage of, or around the catheter. In 2 of these the leakage was from the side with questionable function and therefore of importance, and in the other 2 from both sides. In 3 further cases ureteral catheters could not be passed. The 3 remaining cases were not included because of severe pain or severe pain with nausea and an irregular fall in blood pressure.

Scattered pieces of information might still be gathered by these tests. Leakage and nausea or pain with blood pressure fall could still produce tests that gave information about the ability to concentrate on both sides as well as figures for the minimum filtration rates on the two sides. Only the cases where ureteral catheters could not be passed on either side were totally unsuccessful in the sense that we only had figures for the total function. These incomplete tests have not been used in the above diagrams or tables.

Discussion

The work dealing with the functional pattern of renal artery stenosis has mostly centered around the practical problem

contrasts sharply with the conditions in chronic pyelonephritis.

7. In asymmetric chronic pyelonephritis the kidney with the lower filtration excretes a less concentrated and in relation to the filtration more Na-containing urine.

8. Cases with coincidence of parenchymatous lesions and renal artery stenosis will present a pattern decided by the predominance of either of the two conditions. There are cases in this group where vascular reconstructive surgery might be applied.

Acknowledgements

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duced on the stenotic side and all to a parallel extent.

In acute reduction of B. P (and also during the phase of either return of filtration and plasma flow or prolonged reduction) as well as in forced hydration H_2O Na^+ Cl^- and total CO_2 showed a closely parallel behaviour while filtration plasma flow K^+ PO_4 and NH_3 also presented a parallelism which was clearly separated from the former group

The closely parallel behaviour of K^+ and PO_4 in particular immediately raises the question whether at least some part of the total urinary output of PO_4 is secreted tubularly and thus only indirectly influenced by changes in filtration.

In 40 of their 50 essential hypertensives Baldwin et al. (1 14) found a greater difference between the two kidneys in filtration and sodium output than in the group of normals. Most of these cases showed either an exactly corresponding difference in filtration and Na^+-output between the two sides or a more pronounced lowering of the Na^+-output on the side with the lower filtration. This series was evidently not screened with abdominal aortography However it is inconceivable that 80 % of these cases should have had major renal artery stenosis. The most likely explanation might be irregular distribution of complete closures or incomplete stenosis of smaller branches of the renal arteries. Interesting in this connexion is that Baldwin et al. (1 14) had excluded advanced lesions from their series.

It is important to decide whether a moderate lesion of one or two renal arteries with no gross decrease in filtration and plasma flow but with a definite decrease in the output of Na^+ and H_2O may cause hypertension which may be relieved by adequate surgery A number of border line cases must therefore be thoroughly studied to settle this interesting problem This is one further reason for adequate functional analysis.

The coincidence of renal artery stenosis and primary parenchymatous disease is a practical problem of considerable difficulty These cases seem to illustrate renal artery stenosis as a secondary and aggravating feature in cases where hypertension has most probably another primary cause.

Summary

1 In main stem unilateral stenosis there is a sharp reduction of the excretion of H_2O Na^+ Cl^- and total CO_2 on the stenotic side The reduction in the excretion of K^+ PO_4 and NH_3 is moderate and closely parallels the fall in filtration and plasma flow

2 Acute reduction of B. P usually enhances the difference between the sides. In particular there seems to be a parallel increase in the difference between the two sides as regards filtration, plasma flow K and PO

3 On forced hydration there is a very sharp increase in the output of H_2O, Na^+ Cl^- and total CO_2 on the non-stenosed side, while the stenosed side responds very little and in the case of K^+ and PO_4 not at all.

4 In border line cases with discrete artery lesions on arteriography and equal filtration and plasma flow difference in electrolyte and H_2O output may nevertheless be found.

5 Clearcut patterns are often not found in bilateral renal artery stenosis and in branch lesions.

6 The highly concentrated urine and the retention of Na^+ in the stenotic kidney

contrasts sharply with the conditions in chronic pyelonephritis.

7. In asymmetric chronic pyelonephritis the kidney with the lower filtration excretes a less concentrated and in relation to the filtration more Na-containing urine.

8. Cases with coincidence of parenchymatous lesions and renal artery stenosis will present a pattern decided by the predominance of either of the two conditions. There are cases in this group where vascular reconstructive surgery might be applied.

Acknowledgements

We are indebted to our colleagues of the radiological and surgical departments for their continuous co-operation in this series of cases. For technical assistance we thank Mrs Kerstin Ahlqvist, Miss Sonja Carlsson and Miss Berit Thomson, Miss Mona Carlsson and Miss Ulla Gerrandson and for secretarial assistance Mrs Berit Bengtsson.

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duced on the stenotic side and all to a parallel extent.

In acute reduction of B. P (and also during the phase of either return of fil tration and plasma flow or prolonged reduction) as well as in forced hydration H_2O Na^+ Cl^- and total CO_2 showed a closely parallel behaviour while filtration plasma flow K^+ PO_4 and NH_4 also presented a parallelism which was clearly separated from the former group

The closely parallel behaviour of K^+ and PO_4 in particular immediately raises the question whether at least some part of the total urinary output of PO_4 is secreted tubularly and thus only indirectly influenced by changes in filtra tion.

In 40 of their 50 "essential hypertensives Baldwin et al. (1 14) found a greater difference between the two kidneys in filtration and sodium output than in the group of normals. Most of these cases showed either an exactly corresponding difference in filtration and Na^+-output between the two sides or a more pronounced lowering of the Na^+-output on the side with the lower filtration. This series was evidently not screened with abdominal aortography However it is inconceivable that 80 % of these cases should have had major renal artery stenosis. The most likely explanation might be irregular distribution of com plete closures or incomplete stenosis of smaller branches of the renal arteries. Interesting in this connexion is that Baldwin et al. (1 14) had excluded ad vanced lesions from their series.

It is important to decide whether a moderate lesion of one or two renal arteries with no gross decrease in filtration and plasma flow but with a definite decrease in the output of Na^+ and H_2O may cause hypertension which may be relieved by adequate surgery A number of border line cases must therefore be thoroughly studied to settle this interesting problem This is one further reason for adequate functional analysis.

The coincidence of renal artery stenosis and primary parenchymatous disease is a practical problem of considerable difficulty These cases seem to illustrate renal artery stenosis as a secondary and aggravating feature in cases where hypertension has most probably another primary cause.

Summary

1 In main stem unilateral stenosis there is a sharp reduction of the excretion of H_2O Na^+ Cl^- and total CO_2 on the stenotic side The reduction in the excretion of K^+ PO_4 and NH_4 is moderate and closely parallels the fall in filtration and plasma flow

2. Acute reduction of B. P usually enhances the difference between the sides. In particular there seems to be a parallel increase in the difference between the two sides as regards filtration, plasma flow K^+ and PO_4

3 On forced hydration there is a very sharp increase in the output of H_2O, Na^+ Cl^- and total CO_2 on the non-stenosed side while the stenosed side responds very little and in the case of K^+ and PO_4 not at all.

4 In border line cases with discrete artery lesions on arteriography and equal filtration and plasma flow difference in electrolyte and H O output may nevertheless be found

5 Clearcut patterns are often not found in bilateral renal artery stenosis and in branch lesions.

6 The highly concentrated urine and the retention of Na^+ in the stenotic kidney

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Calcium Deficiency Following Partial Gastrectomy

By

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Partial gastrectomy may lead to defective absorption of certain food components. If this absorption defect involves calcium and/or vitamin D development of osteomalacia must be expected. True, mild radiographic changes (2, 15) and varying degrees of clinical and radiographic osteomalacia (1 7 3) have been reported, although their incidence is unknown, and according to Nicolaysen and Ragaard (9) the calcium balance is often negative in gastrectomized patients.

In view of the increasing number of patients who are nowadays being subjected to subtotal gastrectomy the problem of a possible later calcium deficiency in the skeletal system is becoming an acute one. Radiographic changes do not occur however until an advanced stage of calcium deficiency (5) and it is difficult to carry out time-consuming calcium balance studies on large groups of patients. The present study on a group of partially gastrectomized patients was designed to throw some light on the problem by means of the calcium infusion test advocated by Nordin and Fraser (10)

Material

Our material comprised 38 patients (29 men and 9 women ranging in age from 24 to 70 years) operated upon 6 months to 20 years previously (cf. fig. 6) for gastric or duodenal ulcer. Thirty-three had had Billroth II or Polya resections, while 3 had had the Billroth I procedure. Lastly one patient had had total gastrectomy and one gastro-enteroanastomosis. At the time of the study 26 of the 38 patients were in hospital because of symptoms which could be attributed to the gastric surgery while the remaining 12 were investigated while in hospital for conditions unrelated to the gastric resection.

For comparison, we studied *13 non-operated patients* with gastric or duodenal ulcer (12 men and 1 woman, aged 32—63)

Methods

Calcium infusion test determining the "net 12-hour urinary excretion of calcium"

Our method was that advocated by Nordin and Fraser (10) whose paper may be consulted for details. The principle is as follows

The subject is kept on a low-calcium diet, containing approx. 140 mg calcium daily for four days. The basal urinary excretion of calcium is determined on the third day. On

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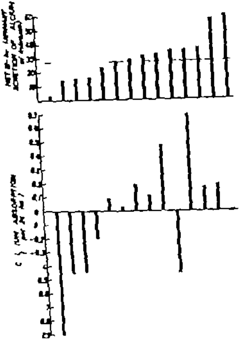


Fig. 2. Corresponding values for "net 12-hour urinary excretion of calcium" and the result of calcium absorption experiments (for details see the text) in 14 partially gastrectomized patients.

Alkaline phosphatases in the serum (fig. 3) were investigated in 26 of the operated patients. The result showed a significant, negative correlation with the "net 12-hour urinary excretion of calcium" (r=0.49, P=0.01).

The vitamin B_{12} absorption (fig. 4) was not correlated to the "net 12-hour urinary excretion of calcium". The Schilling test showed reduced values in 7 out of the 19 patients.

Twenty-two of the 38 patients had *iron-deficiency anaemia*. In these patients the "net 12-hour urinary excretion of calcium" did not differ significantly from that in 16 patients without anaemia.

X-ray studies of the stomach after the operation were available for 28 of the patients. An attempt was made to evaluate the size of the gastric stump. In each individual case, planimetry was performed on the two films which showed the largest and the smallest gastric area, and the mean of these two values was taken to represent the size of the gastric stump. In fig. 5 these results are compared with the "net 12-hour urinary excretion of calcium". It is immediately clear that no correlation exists between these values.

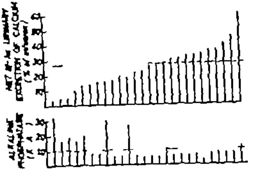


Fig. 3. Corresponding values for the "net 12 hour urinary excretion of calcium" and the serum level of alkaline phosphatases in 26 partially gastrectomized patients.

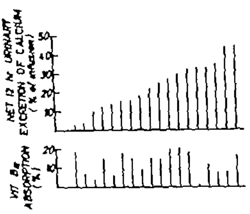


Fig. 4. Corresponding values for the "net 12 hour urinary excretion of calcium" and the vitamin B_{12} absorption determined by the Schilling test in 18 partially gastrectomized patients.

Diarrhoea or radiologically demonstrable jejunitis with a short small intestine transit,

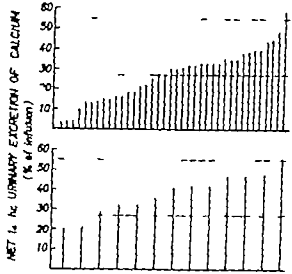


Fig 1 "Net 12 hour urinary excretion of calcium" in 38 partially gastrectomized patients (top) and in 13 non-operated patients with gastric or duodenal ulcer

the fourth day the patient receives an intravenous infusion of a calcium levulinate solution rendered isotonic by NaCl. A total of 15 mg Ca/kg body weight is given in 4 hours. The urinary excretion of calcium is determined during the first 12 hours after the start of infusion. From the result is deducted the basal excretion in 12 hours. This difference is expressed in per cent of the infused dose and called the "*net 12-hour urinary excretion of calcium*"

In Nordin and Fraser's (10) normal series the "net 12-hour urinary excretion of calcium" averaged 41 % (S.D 7 %) the normal range (M ± 2 S.D) being 27—55

In the presence of *osteomalacia* with preserved bony matrix and a preserved ability to take up calcium from the blood, the bones will retain more of the infused quantity than they would normally do, so that the "net 12 hour urinary excretion of calcium" will be reduced. This was elucidated by Nordin and Fraser (10) in patients suffering from osteomalacia, confirmed biochemically radiologically and by skeletal biopsy In *osteoporosis* on the other hand, the bones are unable to deposit calcium, because the matrix is deficient, and the "net 12-hour urinary excretion of calcium" will therefore generally be normal.

Calcium absorption test

For 5 days the calcium intake was fixed at 790 mg daily viz. 140 mg in the diet and 650 mg as lactate in tablet form. The faeces from this period was collected and analyzed for calcium. The calcium absorption was calculated as the average per 24 hours.

Vitamin B_{12} absorption was determined by the method of Schilling (12) The technique has been described in a previous paper (6).

Chemical methods

Calcium analysis of serum and urine was carried out by precipitation as oxalate, dissolution of sediment in HCl, addition of an excess of EDTA, and titration with $MgCl_2$ using Eriochrome black as indicator

Calcium analysis of faeces was performed by permanganate titration after ashing

Alkaline phosphatases in the serum were determined as advocated by Kind and King (4)

Fat in the faeces was determined by a modification of the method described by van de Kamer et al. (14)

Results

Fig 1 sets out the "*net 12-hour urinary excretion of calcium*" in the 38 patients with a history of gastric surgery compared with the 13 non-operated ulcer patients. Out of the 38 operated patients 16 had values below the normal range. Out of the 13 non-operated patients 11 had normal values, 2 slightly reduced values, viz. 20 % and 21 % This difference between operated and non-operated patients is significant ($0.001 < P < 0.01$)

A calcium absorption test was performed on 14 patients (fig 2) In five of them more calcium was found in the stools than had been administered by mouth. The "net 12 hour urinary excretion of calcium" is given for the same 14 patients. There is a statistically significant correlation between the "net 12-hour urinary excretion of calcium" and the calcium absorption ($r = 0.58$, $0.01 < P < 0.05$)

Case 3. A male born in 1889. Since approx. 1940 ulcer complaints leading to gastro-enterostomy in 1950. Since 1955 increasing backache and tendency to collapse resulting in kyphosis. In June 1959 X-rays of the vertebral column showed that all the vertebral bodies were poor in calcium. There was partial collapse of the 11th dorsal and the 1st, 2nd, and 4th lumbar vertebrae and pronounced kyphosis. Serum calcium 8.4 mg/100 ml, serum phosphorus (inorganic) 3.1 mg/100 ml, alkaline phosphatases 16.2 KA units. X-rays of the stomach showed sequelae to gastro-enterostomosis and a short small-intestine transit time. "Net 12 hour urinary excretion of calcium" 4 %.

Discussion

The calcium infusion test determining the "net 12-hour urinary excretion of calcium" as well as the balance studies determining the absorption of calcium from the intestinal tract consistently show that gastric resection may entail a calcium deficiency condition, the low "net 12-hour urinary excretion of calcium" indicating osteomalacia. The present material does not allow any deduction as to the frequency with which such a calcium deficiency is caused by gastric resection. True, almost half the patients had abnormally low values at the calcium infusion test, but the material can by no means be considered representative of gastrectomized patients in general. About half the patients had been admitted to hospital because of dyspepsia, and from one might expect calcium deficiency to be particularly common among such patients. It may be mentioned, however, that a low "net 12-hour urinary excretion of calcium" was found in no less than 5 out of the 12 gastrectomized patients studied during admission to hospital for conditions entirely unrelated to the gastric surgery.

We found a correlation between a low "net 12-hour urinary excretion of calcium" on the one hand and a reduced calcium absorption from the intestine as well as an elevated serum level of alkaline phosphatases on the other, but no correlation to the time interval after the operation. These findings might indicate that a low "net 12-hour urinary excretion of calcium" reflects the activity of the osteomalacic process rather than the actual calcium content of the bones. This renders the 12-hour excretion test well-suited for an early assessment of whether a patient subjected to partial gastrectomy runs the risk of developing a calcium deficiency condition. Moreover, as a screening test the method is easier, quicker and cheaper than a balance study.

It might be contemplated whether the spontaneous 24-hour urinary excretion of calcium on a low-calcium diet could perhaps afford the same information as the calcium infusion test. The finding of a certain conformity between these two tests is only what might be expected, and indeed this is apparent from fig. 7 ($r = 0.63$, $P < 0.01$). In the individual cases, however, there may be a marked discrepancy. All 3 patients with clinical symptoms from the skeletal system had a low "net 12-hour urinary excretion of calcium" (4, 10, and 13 %), while there was a marked variation in the spontaneous 24-hour excretion (4, 80, and 29 mg). In our normal series too, there was a marked scatter in the spontaneous 24-hour excretion of calcium, viz. from 15—180 mg. Correspondingly, Nordin and Fraser (10) found the spontaneous urinary excretion of calcium to be considerably less valuable than the calcium infusion test in diagnosing osteomalacia.

In respect to the pathogenesis of the deficient calcium absorption following

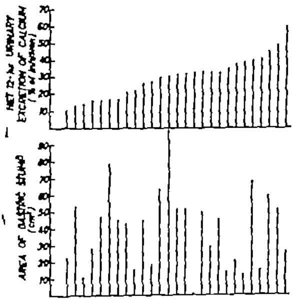


Fig 5 Corresponding values for the "net 12-hour urinary excretion of calcium" and the radiological area of the gastric stump (for details see the text) in 27 partially gastrectomized patients.

tone was found in 15 patients. In these cases the "net 12 hour urinary excretion of calcium" averaged 17 % as compared with 32 % in the remaining 23 This difference is significant (0 001 < P < 0 01) Among the cases of diarrhoea 8 also had gross *steatorrhoea* and in these 8 cases the "net 12 hour urinary excretion of calcium" averaged 16 %

The *time interval from the operation* was plotted against the result of the calcium infusion test, but this did not show any definite correlation in our series (fig 6)

The three patients who had been operated upon by the Billroth I procedure had a "net 12 hour urinary excretion of calcium" of 3 32 and 45 % respectively In the patient who had had gastro-enteroanastomosis it was 3 5 % and in the totally gastrectomized patient 15 % These few observations do not permit an evaluation of a possible correlation between the type of surgery and the tendency to develop calcium deficiency

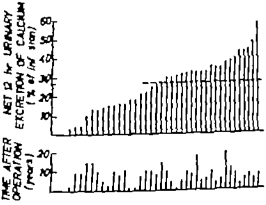


Fig. 6. Corresponding values for the "net 12-hour urinary excretion of calcium" and the time interval after operation in 38 partially gastrectomized patients.

Manifest osteomalacia with subjective complaints and radiographic skeletal changes was present in 3 cases.

Case 1 A male born in 1910. Since 1932 symptoms of ulcer leading to gastric resection (Billroth II) in 1943. Re-operation in 1947 and vagotomy in 1949 because of persistent complaints. After having had increasing backache for some years, the patient noted in 1958 that he had developed slight kyphosis and had got shorter August 1959 X-rays of the spine revealed marked kyphosis and generalized radiolucency Serum calcium 8 mg/100 ml, serum phosphorus (inorganic) 3.0 mg/100 ml, alkaline phosphatases 14.9—19.2 KA units/100 ml. X rays of the stomach and intestine revealed the sequelae to gastric resection and moreover jejunitis. "Net 12-hour urinary excretion of calcium" 10 %

Case 2 A woman born in 1908. Since 1940 symptoms of ulcer leading to gastric resection (Billroth II) in 1949 After the operation weight loss, diarrhoea, and gross steatorrhoea. Since 1956 repeated fractures (metatarsal bones, ribs, radius) occurring spontaneously or following slight injuries. Periodically backache In August 1958 X rays of the spine showed moderate scoliosis with calcium deficiency of the bones. Serum calcium 7 6 mg/100 ml, serum phosphorus (inorganic) 3.1 mg/100 ml, alkaline phosphatases 14.8—19.1 KA units/100 ml, "net 12 hour urinary excretion of calcium" 13 %.

Summary

To assess the risk of calcium deficiency as a sequel to gastric surgery the calcium infusion test used by Nordin and Fraser (10) was performed on 38 patients who had been subjected to gastric surgery (partial gastrectomy in 36 cases, total gastrectomy in 1 and gastroenteroanastomosis in 1) 6 months to 20 years previously. For comparison, 13 non-operated ulcer patients were investigated by the same technique. A low "net 12-hour urinary excretion of calcium" as in osteomalacia was found in 16 of the 38 operated cases, but in only 2 of the 13 non-operated ones. Manifest osteomalacia was found in only 3 of the operated cases.

In 14 of the operated patients the calcium absorption from the intestine was determined on daily administration of 790 mg calcium during a 5-day period. Five patients showed a faecal excretion of calcium exceeding the dose given by mouth. There was a significant correlation between the net 12-hour urinary excretion of calcium and the calcium absorption from the intestine.

A low net 12-hour urinary excretion of calcium was also found to be correlated to a high serum level of alkaline phosphatases. The lowest values were recorded in patients with chronic diarrhoea, steatorrhoea, or radiographic signs of jejunitis and short small-intestine transit time. On the other hand there was no relation to the time interval after the operation or to the size of the gastric stump to vitamin B_1 absorption, or to the presence of iron-deficiency anaemia.

It is concluded that partially gastrectomized patients have a tendency to develop a calcium absorption defect resulting in osteomalacia. The "net 12 hour urinary excretion of calcium" appeared to provide more reliable information than the spontaneous 24-hour urinary excretion of calcium on a low-calcium diet. Using the infusion test, osteomalacia may be demonstrated before it gives rise to clinical and radiographic signs. That the infusion test showed signs of osteomalacia in connection with elevated serum levels of alkaline phosphatases, but independently of the time interval after the operation, indicates that the test reflects the activity of the osteomalacic process rather than the actual calcium content of the bones.

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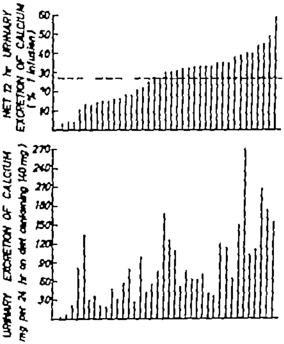


Fig. 7 Corresponding values for the "net 12 hour urinary excretion of calcium" and the spontaneous 24-hour excretion of calcium in the urin on a diet containing 140 mg calcium daily in 38 partially gastrectomized patients.

gastric resection various possibilities suggest themselves. The most important factor may be a deficient reservoir function of the stomach with resulting jejunitis. In our series, patients with radiological evidence of jejunitis had a lower "net 12 hour urinary excretion of calcium" than patients without jejunitis. Another factor to be considered is the deficient admixture with and stimulation of biliary and pancreatic secretion due to the food by passing the duodenum. After Billroth I resection, in which the normal passage through the duodenum is preserved this latter possibility ought not to apply Nevertheless, one of our 3 patients operated upon by the Billroth I procedure had a "net 12 hour urinary excretion of calcium" of only 3 % indicating an extremely active osteomalacic process.

Lastly it must be asked whether the more alkaline environment in these patients digestive tract, with abolished or reduced acid secretion in the stomach, may inhibit calcium absorption, perhaps due to the formation of calcium soaps or of other insoluble calcium compounds. If this factor were important one would expect a correlation between the remaining acid secretion and the absorption of calcium from the intestine. It is difficult, however to carry out a quantitative determination of the acid secretion in gastrectomized patients. In our series, we confined ourselves to comparing the "net 12 hour urinary excretion of calcium" with the size of the gastric stump — without finding any correlation. Furthermore, the only patient treated exclusively by gastro-enteroanastomosis and whose acid secretion was consequently still high, showed clinical as well as biochemical signs of osteomalacia.

Should the achlorhydria be decisive other achlorhydric patients would also be expected to develop osteomalacia. True, Meulengracht (8) has described skeletal changes in elderly women with achlorhydria, but it is doubtful whether this really did represent osteomalacia. Such changes in post menopausal women should more probably be interpreted as osteoporosis.

In our series, a calcium deficiency condition often co-existed with other types of malabsorption. For instance, a number of the patients with a particularly low "net 12 hour urinary excretion of calcium" also had steatorrhoea. On the other hand a calcium deficiency state appeared to occur independently of a defective B_{12} absorption and iron deficiency anaemia, indicating that these types of absorption defect following partial gastrectomy are not necessarily of the same pathogenesis.

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Hypergammaglobulinemia and Thyroid Antibodies

By

Bengt Skanse and Stig-Bertil Nilsson

Thyroid antibodies may be substantially responsible for the increase of the γ-globulin values often found in Hashimoto's disease, despite the absence of any close correlation between antibody titers and γ-globulin levels either in this or other thyroid disorders (10). Skanse and Nilsson (11) therefore assessed the specificity of the tests for thyroid antibodies in patients with different types of hypergammaglobulinemia. They found agglutinating antibodies in 13.5 % of their patients and complement-fixing antibodies in 7.8 %. The incidence of positive agglutination tests was roughly equal in all of the diseases studied except liver cirrhosis, in which it was low. The incidence of positive complement-fixation tests was highest in systemic lupus erythematosus. Other investigators have also studied the incidence of thyroid antibodies in various non-thyroid diseases commonly associated with hypergammaglobulinemia (table I).

Further studies of this problem seemed desirable particularly in view of Waldenström and Winblad's (12) finding of an increased incidence of positive serologic reactions (anticomplement titer, antistreptolysin titer, sensitized sheep erythrocyte agglutination and antistaphylolysin titer) in various diseases accompanied by hypergammaglobulinemia, such as disseminated lupus erythematosus, polyarteritis nodosa, dermatomyositis, purpura hyperglobulinemia, rheumatoid arthritis and cirrhosis of the liver, but not multiple myeloma.

This paper is thus an extension of the studies reported by Skanse and Nilsson (11).

Materials and methods

The series consisted of 330 patients with hypergammaglobulinemia, in which paper electrophoresis showed either a diffuse increase or a narrow band in the γ-globulin region, and 250 healthy subjects (table II). The present series includes all the 141 patients studied previously as well as their controls. Patients with known thyroid diseases were excluded.

The tanned-red-cell (T.R.C.) agglutination and inhibition test was conducted with formolized sheep cells coated with thyro-

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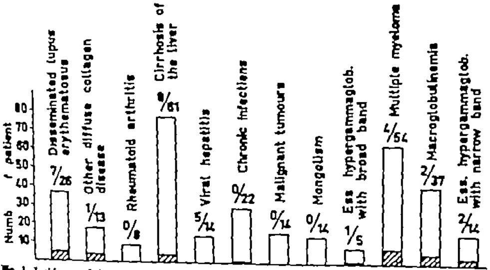


Fig. 1. Incidence of thyroid antibodies in various types of hypergammaglobulinemia. The frequency of patients with positive complement-fixation test is given above each column: this test could not be evaluated in all patients because of gross anticomplementary activity.

Table II. Incidence of thyroid antibodies in diseases with associated hypergammaglobulinemia

Diagnosis	No. of pat.	Sex M	Sex F	Positive serologic tests: T.R.C. No.	T.R.C. %	C.F No.	C.F %	Range of γ-globulins g/100 ml
Disseminated lupus erythematosus	37	3	34	5	13.5	7/26	27.0	1.31—8.25
Other "diffuse collagen" disease	18	2	16	4	22.1	1/13	7.7	1.73—5.12
Cirrhosis of the liver	77	47	30	4	5.2	8/61	13.1	1.30—4.33
Viral hepatitis	14	7	7	0	—	5/14	35.6	1.60—2.49
Chronic infections	29	13	16	1	3.5	0/22	0	1.55—4.80
Malignant tumours	16	8	8	0	0	0/14	0	1.51—3.53
Mongolism	14	9	5	0	0	0/14	0	1.32—2.43
"Essential" hypergammaglobulinemia with broad band	6	3	3	1	—	1/5	—	1.31—3.65
Multiple myeloma	62	34	28	8	12.9	4/54	7.4	1.57—9.64
Macroglobulinemia	40	26	14	5	12.5	*2/37	5.4	1.60—6.95
"Essential" hypergammaglobulinemia with narrow band	15	5	10	3	20.0	2/14	14.3	1.72—2.80
Healthy subjects	230	Mixed		10	4.0	1/230	0.4	

Titres ≥ 1/10 were considered positive.
Titres ≥ 1/5 were considered positive.
The rest of the patients in the group had to be excluded because of anticomplementary activity

Table I Incidence of positive T.R.C agglutination test in diseases associated with hypergammaglobulinemia

Clinical diagnosis	T.R.C. test (titer ≥ 1/10)							
	Hackett et al. (6)		Fahey & Goodman (2)		Mackay & Perry (9)		Hijmans et al. (7)	
	No. of pat.	No. of pos. tests	No. of pat.	No. of pos. tests	No. of pat.	No. of pos. tests	No. of pat.	No. of pos. tests
Disseminated lupus erythematosus	10	3	15	1	33	5	60	15
Other "diffuse collagen" diseases	7	3	—	—	—	—	—	—
Rheumatoid arthritis	10	1	21	1	—	—	72	6
Cirrhosis of the liver	24	5	—	—	31	5	—	—
Dysproteinemia	6	1	14	1	—	—	—	—

[1] Macroglobulinaemia 9 multiple myeloma 2, diffuse hypergammaglobulinemia 3.
[2] Includes also patients with positive complement fixation tests.

globulin (Burroughs Wellcome & Co., London) as described by Fulthorpe et al. (3) In the previous study we used Witebsky and Rose's (13) modification of Boyden's technic (1) for the detection of thyroid antibodies. In order to obtain results comparable with those found in the former study all the positive sera reported in that study were now tested with the commercial preparation. The two tests gave almost identical results. The complement fixing capacity (C.F test) of the sera was estimated with the method of Roitt & Doniach (10) T.R.C. titers ≥ 1/10 and C.F titers ≥ 1/5 were considered positive. In addition, all sera giving positive C.F-reactions with thyroid were tested in parallel using 1/10 saline extracts of human postmortem liver and kidney and 2 M.H.D of complement. The tissue extracts were prepared in the way described by Gajdusek (4)

The paper electrophoretic analysis was performed according to Laurell et al. (8) The normal range for the γ-globulin values was 0.84—1.29 g/100 ml.

Results

The results are summarized in fig 1 and tables II and III The incidence of positive T.R.C. agglutination tests in the diseases studied ranged from 22 % ("diffuse collagen" diseases other than systemic lupus erythematosus) to nil (viral hepatitis malignant tumors and mongolism) The incidence of positive tests was higher than normal in disseminated lupus erythematosus, other diffuse collagen diseases, multiple myeloma and macroglobulinemia but not in cirrhosis of the liver In multiple myeloma and macroglobulinemia, i. e. the two groups with narrow bands, positive tests were about as common as in systemic lupus erythematosus.

The incidence of positive C.F-reactions varied from 27 % (disseminated lupus erythematosus) to nil (chronic infections, malignant tumors, mongolism) (fig 1 and table II) In disseminated lupus erythematosus, cirrhosis of the liver and multiple myeloma it was significantly higher than in the controls. In systemic lupus erythematosus it was definitely more common than in cirrhosis of the liver chronic infections, multiple myeloma and macroglobulinemia. The C.F reaction

Table III (cont.)

Type of disease	Initials, age and sex	γ-glob. g/100 ml	T.R.C. titer	C.F titer	Serum protein-bound iodine μg/100 ml
Multiple myeloma	M.O. 72, M	2.41	1:250	Neg.	7.0
Multiple myeloma	K.H. 60, M	1.57	1:25	Anticomplementary effect	4.9
Macroglobulinemia	A.E. 56, M	3.17	1:25	Neg.	6.5
Macroglobulinemia	J.K. 60, M	4.89	1:2 500	1:10	4.8
Macroglobulinemia	F.O. 73, M	3.76	1:250	Neg.	6.8
Macroglobulinemia	E.G. 67 M	2.80	1:250	Neg.	4.8
Macroglobulinemia	G.W. 63,	5.57	1:2 500	1:5	6.5
Essential hypergammaglobulinemia with narrow band	K.A. 83,	1.91	1:25 000	1:80	7.5
Essential hypergammaglobulinemia with narrow band	H.H. 68, y	2.60	1:250	Neg.	—
Essential hypergammaglobulinemia with narrow band	A.P. 78,	2.04	Neg.	1:10	3.5
Essential hypergammaglobulinemia with narrow band	H.O. 58,	8.58	1:2 500	Neg.	—

could not be evaluated in as many as 66 patients (20 %) because of gross anticomplementary activity (table II).

The incidence of positive C.F reactions was higher than that of positive T.R.C.-tests in systemic lupus erythematosus and cirrhosis of the liver but lower in multiple myeloma and macroglobulinemia.

The groups did not differ in any definite direction from one another regarding titer (table III). That the titers did not appear to vary with the γ-globulin concentration is seen from fig. 2, which includes the results obtained in those patients in whom one or both tests using specific thyroid antigens gave a positive result.

Discussion

Positive T.R.C.-tests and C.F-reactions with thyroid antigen were equally common in the present series of 330 patients with hypergammaglobulinemia accompanying various non-thyroid diseases as in the previous smaller series reported by Skanse and Nilsson (11). In some diseases with associated hypergammaglobulinemia, then, the occurrence of thyroid autoantibodies is too common to be ascribed to chance.

The incidence of positive T.R.C.-tests observed in the present investigation was probably lower than that observed by Hackett et al. (6) but agreed fairly well with the results of Fahey and Goodman (2), Mackay and Perry (9) and of Hijmans et al. (7) (table I). We thus found almost the same frequency in systemic lupus erythematosus as Mackay and Perry and Hijmans et al.

The incidence of positive T.R.C. tests in cirrhosis of the liver was not higher than in the controls. It was thus definitely lower than that reported by Hackett et al. (6) and Mackay & Perry (9) (table I)

Table III Data on patients with positive serologic tests

Type of disease	Initials, age and sex	γ-glob. g/100 ml	T.R.C. titer	C.F titer	Serum protein-bound iodine μg/100 ml
Systemic lupus erythematosus	U B. 27 F	2.84	Neg.	1 20	7.0
Systemic lupus erythematosus	B. P 37 F	1.53	1:250	1:20	6.4
Systemic lupus erythematosus	S. J 28, F	2.12	1 25	*1:10	4.5
Systemic lupus erythematosus	D. S. 50, F	3.15	1:250	1:5	5.9
Systemic lupus erythematosus	G L. 45, F	3.47	1 2 500	Neg	6.5
Systemic lupus erythematosus	E. H. 60, F	1 67	1:250	1:20	4.9
Systemic lupus erythematosus	G. G. 41 F	2.30	Neg.	1:40	7 1
Systemic lupus erythematosus	J K. 46, M	2.13	Neg.	1:10	5.8
Other "diffuse collagen" diseases	E. E. 57 F	2.87	1:25	Anticomplementary effect	6 0
Other "diffuse collagen" diseases	G S. 40, F	3.90	1:250	Anticomplementary effect	8.0
Other "diffuse collagen" diseases	B. L. 32, F	2.53	Neg	1:5	7.8
Other "diffuse collagen" diseases	S. N 56, F	1 73	1 2 500	Anticomplementary effect	5.8
Other "diffuse collagen" diseases	A. G 47 M	1.28	1:250	Neg.	—
Cirrhosis of the liver	A. H. 75, M	2.48	1 25	Neg.	3.4
Cirrhosis of the liver	E. W 58, M	1 49	Neg	1 5	—
Cirrhosis of the liver	S. F 64 F	1 70	1 25 000	1 10	6.4
Cirrhosis of the liver	L. L. 28, F	2.02	Neg.	1 20	7 1
Cirrhosis of the liver	P. M. 33, M	1 42	Neg.	−*1 20	—
Cirrhosis of the liver	H. M. 72, F	1.63	Neg.	1 10	—
Cirrhosis of the liver	J O. 73 M	1 44	Neg.	−*1 20	5.2
Cirrhosis of the liver	G. N. 74, F	1 48	1 25	1 10	5.9
Cirrhosis of the liver	N P 60, M	2.15	Neg.	1 20	—
Cirrhosis of the liver	H. L. 71 F	1 67	1 25	Neg	6.4
Viral hepatitis	K. N 37 M	2.07	Neg	1 20	—
Viral hepatitis	B. E. 12, F	2.00	Neg.	− 1 80	—
Viral hepatitis	J E. 47 M	2.00	Neg	−*1 10	—
Viral hepatitis	A. J 49 F	1.85	Neg.	1:20	—
Viral hepatitis	E. A. 32, F	1.99	Neg.	1 5	—
Chronic infection	A. A. 71 F	1.96	1:250	Neg.	4.9
Essential hypergammaglobulinemia of diffuse type	H. A. 78, F	2.97	1 2 500 000	1 80	5.2
Multiple myeloma	H. N. 70 F	5.67	1 25	Neg.	6.7
Multiple myeloma	I L. 69, M	3.14	1 25	1 10	8.3
Multiple myeloma	H. W 69, F	5.90	1 25	1 5	5.1
Multiple myeloma	H. M. 87 F	4.07	1 2 500	Neg.	6.2
Multiple myeloma	E. S. 61 F	3.36	Neg	1 20	5.1
Multiple myeloma	H. B. 53, M	2.54	1 25	Neg.	6.4
Multiple myeloma	N. J 72, M	3.91	1 250	1 10	5.5

Positive auto-immune complement-fixation with liver antigen (titer 1/10—1/20).
Positive auto-immune complement-fixation with kidney antigen (titer 1/10—1/20).

tiaty though none of the patients showed any symptoms or signs of thyroid disease. The essentially normal serum protein-bound iodine values (table III) also argue against the presence of long-standing Hashimoto's disease in which hypothyroidism often is present. The negative complement-fixation tests with liver or kidney antigens (table III) in almost all patients with the exception of those with viral hepatitis suggest that at least most of the positive reactions were caused by specific thyroid antibodies. The autoimmune complement fixation (A.I.C.F.) reaction in systemic lupus erythematosus was much less often positive than in the series of *Hijmans et al.* Whether this discrepancy may be due to differences in the preparation of antigens — which may be possible although the technic of Gajdusek (4) was used in both investigations — or to some other factor cannot be stated at present.

A possible explanation for the positive serologic tests would be the assumption of subclinical lymphadenoid goiter in certain persons with hypergammaglobulinemia *and a very sensitive antibody* forming apparatus. If so even minute amounts of antigens normally producing *no* reaction might be sufficient to stimulate antibody production with immune cellular infiltration of the thyroid in such persons.

It is noteworthy that positive serological tests for thyroid antibodies were equally common in multiple myeloma and in macroglobulinemia. This finding suggests that the type of *γ-globulin* disturbance is of less significance for the appearance of thyroid antibodies. On the other hand, the *incidence of* positive serological tests varied widely among the various diseases associated with a more general increase of the normal (7 S) γ-globulins, which suggests that factors other than the type of γ-globulin disturbance are also of significance for the development of thyroid antibodies.

It is noteworthy that the T.R.C.-test and the C.F.-reaction probably behaved differently in patients with a more or less generalized increase of the γ-globulins (systemic lupus erythematosus and cirrhosis of the liver) than in the patients with an increase of a single γ-globulin component (multiple myeloma and macroglobulinemia). In the former group the incidence of positive T.R.C.-tests was lower than that of C.F.-reactions in the latter type, it was the reverse. This observation suggests that these two types of hypergammaglobulinemia may differ in the amount of antigens available or in the immunologic response to the antigens. Such differences in the production of certain γ-globulin components may then affect the various thyroid antibodies differently. This may give reason to assume that the various serologic activities are related to different tissue substances and that the tissue substances themselves might dictate at least to some extent, whether the antibodies against them are 7 S or 19 S γ-globulins. It is noteworthy that immunoelectrophoretic analysis of the multiple myeloma series revealed that thyroid antibodies may be present both in the β_{2A} and the γ-type.

It is not known whether the increased incidence of thyroid antibodies in certain diseases with hypergammaglobulinemia *is due to a leakage of* antigen from the thyroid or to an abnormally intense response to minute amounts of thyroid antigens normally not producing an immunologic response. The possible disturbance of cell permeability in "diffuse collagen" diseases would favor the assump-

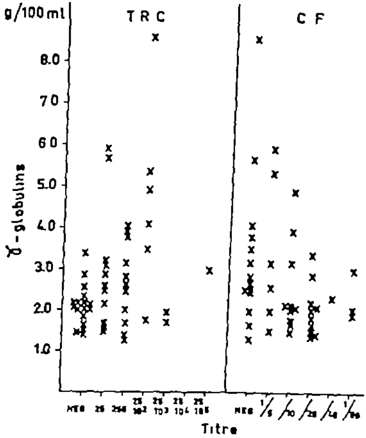


Fig. 2. Relation between thyroid antibody titers (all patients being positive with at least one of the tests are included) and the γ-globulin values.

This discrepancy may at least partly be explained by the greater sensitivity of the T.R.C. test used by these investigators since they found an incidence of 9 % among blood donors, against only 4 % in our group of healthy subjects. The incidence of positive C.F reactions in our series of liver cirrhosis was definitely higher than normal, which is in agreement with the findings of Goudie et al. (5) In contrast to Goudie et al. who found the C.F test to be positive particularly often in elderly females with liver disease no correlation was demonstrable between the results of the C.F test and age or sex in our series. The positive serological tests were about evenly distributed among the various etiologic types of cirrhosis and not, as might perhaps be expected, limited to cryptogenetic cirrhosis, i. e. a group possibly including patients with lupoid cirrhosis.

The titers did not appear to vary with the type of disease (table III) or with the degree of hypergammaglobulinemia (fig 2)

Hijmans et al. (7) found thyroid antibodies in systemic lupus erythematosus in females only This apparent lack of positive tests in males might be ascribed to the rarity of this disease in the male sex. This assumption is supported by our finding of a positive test in one of the 3 males in our series of systemic lupus erythematosus.

Why thyroid autoantibodies should occur in non-thyroid diseases is difficult to explain. Whether the finding of positive serologic tests depends on some previous or existing disorder of the thyroid gland cannot be excluded with cer

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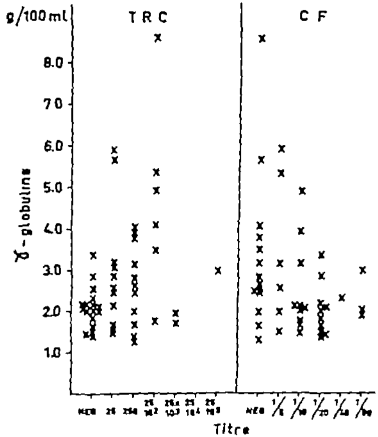


Fig 2 Relation between thyroid antibody titers (all patients being positive with at least one of the tests are included) and the γ-globulin values.

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Complications and Causes of Death in Polycythaemia Vera

By

EVA CHIEVITZ and TORBEN THIEDE

In order to evaluate the complications of polycythaemia vera, their nature and incidence, as well as the causes of death in this disease we collected a series of 250 patients who died during the period 1933—1961. This material was collected from most medical departments in Denmark. Case records, including the older ones, were perused to compare the results of the various therapeutic methods and to procure a group of untreated cases for comparison. All cases in which there might be suspicion of secondary polycythaemia due to hypoxia in the presence of cardiac or pulmonary diseases were excluded.

In Denmark Videbæk (7) has reported on 125 cases. Seventy-five died, 30 % of haemorrhage, 20 % of thrombosis, and 18 % of chronic pyelonephritis. Only 2 succumbed to leukaemia.

In collecting our material we avoided including the cases which made up Videbæk's series.

Wasserman and Bassen in 1959 (6) mentioned the most common causes of death, which were in the following order: Thrombosis, myeloid metaplasia, leukaemia, haemorrhage. He reported that the incidence of leukaemia ranged from 12 % to 80 %.

Present series

In each individual case we traced the disease as far back as possible by looking through the case records from all admissions to hospital. Characteristically the great majority of patients had been hospitalized several times, usually because of the severe complications, so that there was ample opportunity to watch the course of the disease. In some cases we were provided with valuable information by the general practitioners. Thus, the majority of patients were followed for a number of years, and as a rule it was possible to ascertain the approximate time of the first symptom or sign and thus determine the duration of the disease — which we take to be the period from the first symptom or sign until death.

Out of the 250 patients 152 were men and 98 women. The age at onset was as shown in table I. It will be seen that in 118 cases the disease set in between 50 and 65 years of age, in 30 (12 %) before the age of 40, and in 17 (7 %) not until after 75.

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tion of leakage, into the circulation, of normally inaccessible antigens. However such a mechanism appears less likely in multiple myeloma and macroglobulinemia. In these types of disturbances some defect of the antibody forming cells would instead appear to be more likely particularly since the normal γ-globulin fractions are often decreased. On the other hand, the antithyroid reactivity of the serum in patients with hypergammaglobulinemia may be entirely irrelevant, for these conditions are known to be accompanied by an excessive production of globulins with a broad immunological affinity for human tissue proteins. The serologic reactions may also represent a comparatively non-specific protein protein interaction between a tissue component and a serum globulin altered by a non immunologic process. Further studies of this problem are desirable.

Summary

The incidence of thyroid antibodies demonstrable by the tanned red-cell agglutination (T R.C.) test and the complement fixation (C.F.) reaction, was investigated in 330 patients with hypergammaglobulinemia but without demonstrable thyroid disease chiefly patients with systemic lupus erythematosus, other "diffuse collagen diseases" cirrhosis of the liver multiple myeloma and macroglobulinemia

The incidence of positive T R C. tests was higher than normal in systemic lupus erythematosus, other diffuse collagen diseases, multiple myeloma and macroglobulinemia, but not in cirrhosis of the liver

The incidence of positive C.F.-reactions was significantly higher than normal in systemic lupus erythematosus, cirrhosis of the liver and multiple myeloma.

The total incidence of positive serological tests was of the same magnitude both in the groups of patients with a diffuse increase of the γ-globulins and in *those with a narrow band in the γ-globulin* region. Positive serologic tests were observed both in male and in female patients. No correlation was found between the γ-globulin concentration and the titer of the serologic tests.

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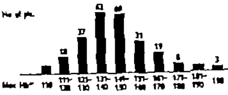


Fig. 1. Maximum Hb% during the disease.

We have plotted the duration of the disease against the form of treatment. Fig. 2 shows the percentage survival time for patients in the various treatment groups.

As is seen from the figure, half the patients of the untreated group had died 18 months after the first manifestation.

X-ray treatment resulted in a survival of more than 12 years in 50 %, while half of all patients treated with venesection only had died within 3 / years.

The corresponding curve for patients treated with P32 and chlornaphazine gives false picture. This treatment is so new that many adequately treated patients from the time of its introduction in 1948 and later are still alive. This curve, therefore, does not represent the average result of this treatment.

The most striking impression of these curves is the poor result of venesection therapy. It seems reasonable to assume that repeated venesections stimulate the bone marrow to further haemopoesis.

As standard of therapeutic efficacy we have also taken the incidence of deaths from thrombosis in the various treatment groups. Among untreated patients 63 % died of thrombosis (50 patients) among those treated with venesection 52 % (52 patients) while only 21 of the X-ray irradiated and 35 % of the P32 and chlornaphazine-treated patients died of this complication. Thus venesection is also not suited for preventing thrombosis.

Forty-one patients were treated with anticoagulants. The majority were treated for only few weeks. Eleven succumbed to thrombosis during the anticoagulant medication. In addition, one patient died of haemorrhage during postoperative anticoagulant medication. Twenty-two patients have died later of repeated thrombosis or emboli, 3 of them within the first year after discontinuation of anticoagulation.

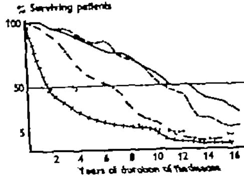


Fig. 2. Percentage survival time for patients in various treatment groups.

various combinations with X-ray therapy.
—— X-ray therapy
P32 and/or chlornaphazine.
venesection.
++++ untreated.

These findings establish that the treatment with anticoagulants must be continued for a long time as prophylaxis against recurrent thrombosis. Despite the bleeding tendency of polycythaemic patients, anticoagulant treatment with frequent control is easily practicable.

Causes of death and complications

In table III the causes of death are listed.

Thrombosis 40 % of the patients died of thrombosis, the most common complication to polycythaemia vera. The sites of the thromboses are shown in table IV

A total of 38 patients died of cerebral thrombosis, 16 after several episodes. The number of deaths from cerebral thrombosis was 5 times that in the general population (15 % as compared with 3 %) when compared with the number of deaths in the adult population over 35 years of age (5) It should be emphasized that a total of 79 patients (32 % of the entire series) had cerebral thrombosis during the course of their disease.

Table I Age at onset of first symptom or sign

Age	< 40	40—44	45—49	50—54	55—59	60—64	65—69	70—74	75—79	80—84
Men	23	11	10	24	26	22	18	9	7	2
Women	7	4	6	15	14	17	14	13	8	0
Total	30	15	16	39	40	39	32	22	15	2

Table II First symptom or sign

	No. of patients
Dizziness	28
Headache	5
Itching	7
Dysbasia	6
Paraesthesiae	1
Dyspepsia	24
Angina pectoris	8
Polycythaemic colouring	35
Splenomegaly — splenic pain	17
Gout	10
Thrombophlebitis of the extremities	19
Cerebral thrombosis	28
Coronary thrombosis	7
Epistaxis or gingival bleeding	11
Haematuria	1
Haemoptysis	2
Suggillations	2
Haematemesis-melaena	13
Accidental finding	[illegible]6

Table II gives the nature and incidence of the first symptom or sign. In 54 cases the initial sign was thrombosis. Thus, this severe complication occurred before any treatment could be started. In 29 cases the first sign was bleeding. These 2 groups make up one-third of the entire series.

Blood values

The characteristic feature of polycythaemia vera is the increased circulating blood volume. The high Hb level is most striking. Fig. 1 shows the distribution of the maximum Hb % in all the patients. In most cases it was 130 —150 % (19.2—22.2 g). Eight patients had values exceeding 180 % (26.7 g) and three 200 % (29.7 g) or over. On the other hand, 7 patients had 110 % (16.3 g) or lower. However, there is no absolute relation between the Hb level and the blood volume, so that the severity of the disease cannot be estimated on the basis of the Hb level in all cases. Another characteristic is the low ESR, usually 0—1 mm. Even in presence of infections the ESR remains low. One patient, for instance, died of widespread miliary tuberculosis with an ESR of 3 mm four days before death. At the same time the Hb level was 63 % (9.3 g), and autopsy showed hyperplastic bone marrow. The white blood picture was extremely varied. Before the treatment half the patients had elevated W.B.C. (exceeding 10 000/µl). A total of 54 patients had immature white cells in the peripheral blood, indicating that increased myelopoiesis co-existed with increased erythropoiesis. High W.B.C. and the presence of immature cells were far more common in patients with splenomegaly (117 patients or 47) probably indicating extramedullary haemopoiesis. A total of 51 patients had white cell counts exceeding 20,000/µl.

Treatment

The chief forms of treatment were as follows

No treatment	49 patients	
Venesection alone	62 »	
X rays alone	48 »	(24 received high doses)
P^{32} and/or chlornaphazine	52 »	(14 received high doses of P^{32} only)
X rays combined with mitostatics	36 »	(20 received high doses of X-rays).
Chlormethamine	3 »	

ties. The remaining three were suffering from dyspepsia, but X-rays did not show gastric ulcer

(b) Five patients died of large suggilations or haematomas, only one of which was caused by severe injury

(c) Five died of postoperative haemorrhage.

(d) Only one patient died of multiple haemorrhages diagnosed as haemorrhagic diathesis.

The deaths from haemorrhage do not include 8 patients who died of cerebral haemorrhage some of them had normal blood pressure.

In 68 cases haemorrhage occurred during the course of the disease. Thirty four patients had haematemesis and another 11 melaena only Of these patients 18 had gastric ulcer confirmed by radiography

Three patients had cirrhosis of the liver with oesophageal varicosities. Eight had macroscopic haematuria, 22 major cutaneous haemorrhages, and one haemarthros. Haemorrhagic diathesis with multiple haemorrhages occurred in 5 cases, and 26 had epistaxis or gingival haemorrhages.

In other words, haemorrhage was somewhat less common than thrombosis. The same patient would often have haemorrhage as well as thrombosis. We could not find any correlation between the platelet counts and haemorrhage or thrombosis.

Leukaemia, myelofibrosis and anaemia. Twenty five patients succumbed to these diseases. This group will be discussed later

Portal cirrhosis. Three patients died of hepatic failure A total of 7 patients were suffering from cirrhosis. A history of abuse of alcohol was obtained in only one Six of these patients had clinically demonstrable splenomegaly 4 had oesophageal varicosities and of the latter 3 had haematemesis.

Urinary tract disease Twenty patients died of urinary tract diseases, including 11 who succumbed to pyelonephritis causing pyonephrosis in 2. Three died of cancer· One of renal adenocarcinoma and one of bladder cancer These two patients will be reported in more detail in the section on therapy The third patient was presumably suffering from hypernephroma. The primary tumour was not demonstrated but microscopic examination of metastases in the bones and lungs indicated hypernephroma. This is the only case of hypernephroma in our series of 250 patients. In 5 cases the cause of death was given as uraemia. Two of these patients had diabetes mellitus. One patient with nephrolithiasis died in his home the diagnosis was anuria.

A total of 75 patients had urinary tract disorders (30 %) Pyelonephritis was most common (present in 31 patients or 12 %) Data regarding renal calculi were given in 33 cases, all of whom had elevated serum concentration of uric acid. A total of 12 patients developed uraemia. Hydronephrosis was present in 2 In addition to the tumours mentioned above as causes of death, there was yet another case of bladder cancer and one of bladder papilloma. As for benign tumours of the kidney one patient had leiomyoma and one Boeck's sarcoid with renal infiltrations. Cystitis was a common finding

Heart diseases. In addition to the above mentioned 27 patients who died of coronary thrombosis, 21 died of other heart diseases. Moreover 49 patients had cardiac signs during the course of their disease, often angina pectoris. Twenty-one had non-fatal coronary thrombosis.

Table III Causes of death

	No. of patients
Thrombosis or emboli	100
Haemorrhage	15
Leukaemia myelofibrosis-anaemia	25
Cirrhosis of the liver	3
Urinary tract diseases (incl. cancer in 3)	20
Heart diseases (excl. coronary thrombosis)	21
Cancer (excl. cancer of the urinary tract)	9
Bronchopneumonia	14
Cerebral haemorrhage	8
Senility-debility	6
Accidents, suicide	7
Postoperative	5
Miscellaneous	7
Uncertain or unknown cause	10
Total	250

Coronary thrombosis was the cause of death in 18 male and 9 female cases. A total of 48 patients (19 %) had coronary thrombosis. The sex and age distribution corresponded to that in Holten's (1) material of patients with coronary thrombosis.

In addition to 13 patients who died of pulmonary emboli or infarction (5 % as compared with 0 4 % in the general adult population) 16 patients had pulmonary infarction as a complication.

A characteristic feature is mesenteric thrombosis which was the cause of death in 11 cases (4 %) 7 women and 4 men. Five of the patients were under 55 years of age.

Nine patients succumbed to thromboses of the limbs, usually in the femoral artery or vein. A total of 11 patients developed gangrene of an extremity but it must be mentioned that 2 of these patients had diabetes mellitus. Four patients had amputation of the leg A total of 72 patients had extremity thrombosis in the course of their disease.

Table IV Deaths from thrombosis

	No. of patients
Cerebral thrombosis	38
Coronary thrombosis	27
Pulmonary embolism or infarction	13
Mesenteric thrombosis	11
Extremity thrombosis	9
Generalized thrombosis	2
Total	100

Two men died of generalized thrombosis

Example Finsen Institute. A 74-year-old man was referred in October 1957 because of polycythaemia. In April 1957 he had had thrombosis of the left radial artery in April 1958 he developed cerebral thrombosis, and he died in July 1959 Autopsy showed thrombophlebitis of both femoral veins, the inferior vena cava, and the left axillary vein, emboli of the pulmonary artery infarct in the right lung

A total of 158 patients (63 %) had thrombosis. During the course of their disease many patients had several varieties of thrombosis. Fourteen patients had both cerebral and coronary thrombosis.

Example Aarhus Municipal Hospital. In the case of a 54-year-old man polycythaemia vera was diagnosed in 1948 after he had been suffering from headache, dizziness, and blurred vision for a couple of years. B.P 145/70. In 1949 he had thrombosis of the left leg, in 1950 coronary thrombosis, in the same year cerebral thrombosis, in 1957 gout, in 1959 duodenal ulcer and cerebral thrombosis, and in 1960 cerebral thrombosis for the third time. The patient died in January 1961 of thrombophlebitis of the right femoral vein with gangrene of the right foot.

Haemorrhages Fifteen patients died of hemorrhage.

(a) Four patients died of haematemesis one of these had cirrhosis of the liver and died of bleeding from oesophageal varicos-

Myelofibrosis-leukaemia-anaemia

In the great majority of the 250 patients erythropoiesis was dominating at the onset of the disease, judging by the peripheral blood picture as well as the bone marrow. A number of patients showed, usually after a duration of several years, a falling Hb level and a leukaemoid reaction. In some of these cases the polycythaemia passed gradually or suddenly into leukaemia. Some had been followed at regular intervals by bone-marrow studies from which the gradual development of myelofibrosis could be watched — from maximum hyperplasia of the bone marrow with islets of myelofibrosis via predominant myelopoiesis, to a gradually sparse hypoplastic bone marrow. Concurrently there was marked extramedullary haemopoiesis, corresponding to the clinically demonstrable splenomegaly and gradually also hepatomegaly. Autopsy also showed in some cases haemopoiesis in the lungs, kidneys, and lymph nodes.

Eleven patients died of myelofibrosis. Of the 6 patients who died of severe anaemia 3 died in a state reminiscent of myelofibrosis, but as no investigation of the bone marrow had been carried out at the time when anaemia set in, it was impossible to arrive at a more exact diagnosis.

A total of 14 patients developed myelofibrosis of these 9 were males and 5 females. At the time of death, 4 patients were under 55 years of age. It was a characteristic feature that the polycythaemia had been present for a number of years before the onset of myelofibrosis, in 8 cases for more than 10 years. The duration of the myelofibrosis proper (from the time it was diagnosed on the basis of bone-marrow findings until death) ranged from a few months up to

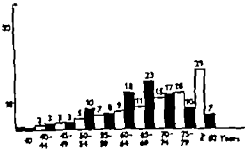


Fig. 3. Age at death.
■ = present series.
□ = general population over 35 years of age at death.

6 years. Only 4 patients had white blood counts below 10 000/μl at the onset of polycythaemia.

Myeloid leukaemia occurred in a total of 9 cases and was the cause of death in 8. The 9th patient died from thrombosis of the portal vein. In 3 its onset was acute and its course short and hyperpyretic. Autopsy showed in all 3 cases "blast" leukemia with numerous, large undifferentiated cells in the bone marrow. In the remaining 6 the leukaemia developed slowly, the white blood count increasing steadily while at the same time the Hb level dropped.

Lawrence (2) found 16 cases of leukaemia and one of myelofibrosis among 73 deceased polycythaemic patients.

Stroebel and Law (4) described 8 cases of acute leukaemia and 9 of an anaemic leukaemoid stage out of 39 fatal cases from among a series of 241 polycythaemics all treated with P^{32}.

Ledlie (3) has reported on 21 deaths among 117 patients treated with P^{32}. Among these 21 there were 5 cases of acute leukaemia and one of chronic myeloid leukaemia. The dosage in these cases had ranged from 11 to 59 mC. The leukaemia had come on 2 $\frac{1}{2}$—4 years

Fifty-seven patients had arterial hypertension This incidence is not striking when the patients age is taken in consideration

The ECG showed apart from the changes of coronary thrombosis very few abnormalities and in 64 cases it was normal Left sided axis deviation was found in 131 cases.

Cancer In addition to the 3 patients who died of cancer of the urinary tract another 9 died of malignant tumours. The following tumours were recorded as the cause of death

- 2 cancers of the stomach
- 1 cancer of the gallbladder
- 1 cancer of the sigmoid colon
- 1 cancer of the lung
- 1 cancer of the kidney (adenocarcinoma)
- 1 cancer of the urinary bladder
- 1 cancer of the prostate
- 1 case of hypernephroma
- 1 case of malignant glioma
- 2 cases of lymphosarcoma

In other words, 12 patients died of malignant tumours. This is 5 % of the 250 cases

Malignant tumours were present in another 7 patients

- 2 with cancer of the face (treated, no signs of recurrence)
- 1 with cancer of the larynx (treated, no signs of recurrence)
- 1 with testicular seminoma (treated no signs of recurrence)
- 1 with cancer of the uterine cervix, stage II (treated, no signs of recurrence)
- 1 with cancer of the body of the uterus (adenocarcinoma, found at autopsy)
- 1 with cancer of the urinary bladder

Twelve patients had other tumours, of these 8 had uterine fibromyomas.

The incidence of malignant tumours in this series is no higher than among the general population, when the patients' age is taken into consideration. Special factors apply to the urinary tumours.

Accidents suicide These were the causes of death in 7 cases. Three patients committed suicide and four were killed in accidents

Postoperative deaths Two patients died of paralytic ileus and one of perforation of the stomach following operation for peptic ulcer Two patients went steadily downhill after the operation. In addition to the named deaths, 5 patients succumbed to postoperative haemorrhage, and of these 5 only one was on anticoagulant therapy

Miscellaneous causes of death. These include One patient who died of gout from which he was completely disabled In this case, the cause of death may be interpreted as a complication to polycythaemia

Two patients died of sepsis and one of miliary tuberculosis. One died of basal arachnoiditis several years after operation for cerebral angioreticuloma. One patient died of ostitis and one of perforated jejunal ulcer The aetiology was unknown.

Unknown or uncertain cause This group comprises 10 patients for whom death certificates could not be procured or for whom the cause of death was merely recorded as cardiac paralysis and there was no possibility of gleaning further data

Age at death. Statistics on the deaths among the population group over 35 years of age in 1959 (5) show the distribution given in fig 3 The percentage is evenly increasing up to the 80th year while in our material the largest number of deaths (23 %) occurred in the age group 65—69 years In all age groups between 50 and 70 there was a definite excess mortality in the case of the patients with polycythaemia, whereas the mortality in the age groups 35 to 50 was the same as in the general population.

Myelofibrosis-leukaemia-anaemia

In the great majority of the 250 patients erythropoiesis was dominating at the onset of the disease, judging by the peripheral blood picture as well as the bone marrow. A number of patients showed, usually after a duration of several years, a falling Hb level and a leukaemoid reaction. In some of these cases the polycythaemia passed gradually or suddenly into leukaemia. Some had been followed at regular intervals by bone-marrow studies from which the gradual development of myelofibrosis could be watched — from maximum hyperplasia of the bone marrow with islets of myelofibrosis, via predominant myelopoiesis, to a gradually sparse hypoplastic bone marrow. Concurrently there was marked extramedullary haemopoiesis, corresponding to the clinically demonstrable splenomegaly and gradually also hepatomegaly. Autopsy also showed in some cases haemopoiesis in the lungs, kidneys, and lymph nodes.

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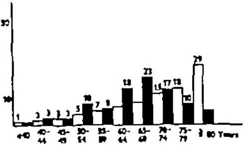


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after the first dose of radioactive phosphorus.

All our 14 cases which ended in myelofibrosis had received intensive treatment usually with large doses of X rays (a total of 10 patients) The remaining 4 had received P^{32} in doses of from 8 to 13 mC in most cases combined with chlornaphazine in some periods.

Seven of the 9 patients who developed leukaemia had also been treated with large doses of X rays 3 of them also with large doses of P^{32} One patient had been treated with P^{32} alone (a total of 14.3 mC) another one had 7.5 mC P^{32} and for several years chlornaphazine.

The most common theory has been that polycythaemia gradually 'burns itself out' ending in an anaemic phase if the patient survives for a sufficient length of time (6) According to the theory this phase will come on regardless of the treatment given, provided that the patient has not already succumbed to complications

A comparison of the various treatment groups in our material, however shows an essential difference in the incidence of myelofibrosis-leukaemia.

For instance, myelofibrosis or leukaemia did not develop in any of the 111 patients who were untretaed or only treated by venesection, although the duration of polycythaemia exceeded 5 years in 36 of these patients. Fifteen were even alive 10 years after the first sign.

In two cases, however the treatment has probably not had any influence on the development of myelofibrosis

In one of these cases the bone marrow showed signs of myelofibrosis 2 years before X ray irradiation had been instituted.

In the other case the sternal bone marrow showed occasional fibroblasts at the time that X ray irradiation was started, and one year later incipient myelofibrosis. So these two cases seem to have developed spontaneously

On the other hand 9 out of 24 patients developed leukaemia or myelofibrosis (37 %) after treatment with large doses of X rays.

Out of 20 patients treated with heavy X ray irradiation combined with other treatment (phenylhydrazine, P^{32} or chlornaphazine) 6 patients (30 %) developed myelofibrosis or leukaemia.

It would therefore, seem beyond doubt that X ray therapy may be a factor in the development of myelofibrosis-leukaemia.

Fourteen patients were treated exclusively with large doses of P^{32} (more than 5 mC) intravenously Among these patients there were 2 cases of leukaemia or myelofibrosis.

A total of 29 patients were treated with large doses of P alone or combined with chlornaphazine and allied drugs or very small doses of X rays. Leukaemia or myelofibrosis occurred in 6 of these 29 patients.

Seven patients had large doses of P^{32} as well as of X rays and 5 developed myelofibrosis or leukaemia (71 %) So this combination gave rise to the relatively largest number of cases of myelofibrosis or leukaemia.

The differential diagnosis between leukaemoid reaction myelofibrosis, and myeloid leukaemia gives rise to great difficulties, as the clinical appearances are usually the same. The blood picture of elevated white cell counts and immature cells is also the same in the early stages of myelofibrosis and leukaemia. Even in the terminal stage 5 patients with myelofibrosis had leukocyte counts exceeding 20 000/µl, a maximum of 78,000/µl. Examination of the bone marrow is neces-

sary and often has to be repeated in order to follow the developments.

Six patients died in a condition diagnosed as anaemia. In 3 of these, a more exact diagnosis could not be made, since there had been no examination of the bone marrow in the terminal stage and no autopsy. None of the patients had bleeding episodes. One of them had 8 years previously been treated with large doses of X-rays and another one with P[32] 6.6 mC 3 years previously.

The other 3 cases of anaemia could be related to the treatment, respectively with TEM, pyrimethamine, and chlornaphazine.

In both the first two cases there was presumably a question of a toxic action upon the bone marrow with a maturation defect.

The third case was due to overdosage of chlornaphazine.

Side effects of chlornaphazine

As a mitostatic chlornaphazine exerts its main action upon the bone marrow, gastro-intestinal mucosa, and the epithelium of the urinary tract. Its side effects are, therefore, dyspepsia and cystitis, often with haematuria.

In our material 26 patients had been treated with large doses of chlornaphazine, i.e. 500 mg daily or more through a minimum period of 3 months. Of these 26 patients 3 developed cancer of the urinary tract.

One developed cancer of the urinary bladder after 2 / years intermittent treatment with chlornaphazine. This patient died at the age of 61 one year after chlornaphazine had been withdrawn.

Another patient died at the age of 54 (in May 1960) of bladder cancer with metastases after having suffered from polycythaemia for 23 years. She was first treated with generalized X rays, then with P[32] a total of 4 mC in two divided doses, during the period 1955—1956. During the period March 1957 to Dec. 1959 she was treated with chlornaphazine 100 mg 3—4 times daily. In 1955 cystitis, and during the treatment periodically some urinary frequency. The urine showed neither albumin nor blood and microscopic examination revealed only 1 or 2 white cells per microscopic field. During the chlornaphazine therapy the patient was seen at short intervals. In Nov. 1959 she was admitted to hospital and in Dec. had an operation for cancer of the bladder (cystectomy). She then went downhill and died in May 1960. Autopsy disclosed metastases in the adrenals and bones as well as polycythaemia.

The third patient died at 70 years of age after having had polycythaemia for 5 years. He had been treated with venesection a few times and with chlornaphazine, 100 mg four times daily for 2 years. Death occurred one month after this treatment had been discontinued. Autopsy revealed adenocarcinoma of the left kidney with metastases to the lungs and a hyperplastic bone marrow.

It is not unlikely that these 3 malignant tumours of the urinary tract may have been due to the large doses of chlornaphazine through long periods. During this therapy, therefore, one must be on the lookout for urinary symptoms. Probably chlornaphazine should not be given to patients with urinary tract complaints and it should be withdrawn in the event of dysuria or haematuria, not to be resumed until the urinary tract has been thoroughly investigated.

None of the patients with cancer of the digestive tract had received chlornaphazine therapy. In one case this treatment

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Summary

The authors review a series of 250 patients with polycythaemia vera from 1933—1961. The course of the disease was elucidated by looking through the case records from all admissions to hospital.

The causes of death are analysed. Forty per cent died of thrombosis, occurring in a total of 158 patients (63 %). Cerebral thrombosis was most common. Haemorrhage was less common. A total of 14 patients developed myelofibrosis, 9 leukaemia, and 6 patients other forms of anaemia.

The results of the various methods of treatment are compared. Out of the untreated patients 50 had died 18 months after the onset of the first symptom or sign, and out of patients treated with venesection half had died 3 / years after this juncture. Out of patients treated by X-rays half were alive 12 / years after the first sign. As yet, it is difficult to evaluate the treatment with P^{32} and chlornaphazine, because the observation period is still short.

The differential diagnostic difficulties in dealing with leukaemia myelofibrosis-anaemia re mentioned. The role of treatment in the development of meylofibrosis and leukaemia is pointed out. Out of 7 patients treated with large doses of X-rays as well as P^{32} 5 developed myelofibrosis or leukaemia.

Out of 26 patients treated with large doses of chlornaphazine 3 developed cancer of the urinary tract.

Out of other complications, gout, nephrolithiasis, and gastric or duodenal ulcer are mentioned.

In order to reduce the number of side effects, it is recommended to combine P^{32} with periods of chlornaphazine and to follow the patients carefully throughout life.

Acknowledgements

It is great pleasure to acknowledge our indebtedness to the heads of the various medical departments in Denmark, general practitioners, and to the Public Records Office (Rigsarkivet). Without their help and courtesy this work could never have been done. Our thanks are due also to Dr Børge Chr. Christensen whose long-continued studies on polycythaemia prompted us to undertake this investigation.

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was followed by severe gastritis One patient developed haematemesis and one melaena on chlornaphazine therapy In two cases the treatment had to be abandoned because of bleeding tendency

Other complications

Gout was a common complication, occurring in 33 cases (13 %) 27 males and 6 females All had elevated values of serum uric acid Several of these patients were totally disabled by this complication having chronic polyarthritis with frequent exacerbations.

Gastric or duodenal ulcer Dyspepsia was a common incipient symptom (cf table II) A total of 51 patients (22 %) had dyspeptic complaints 38 males and 16 females Of these 35 showed X ray evidence of peptic ulcer (25 males and 10 females) but X ray examination had not been carried out in all cases

The incidence of peptic ulcer was higher in this series than in the general adult population (14 % as compared with 7 %)

Other diseases

Severe obesity was present in 15 cases diabetes mellitus in 10 Seven patients had goitre including one with thyrotoxicosis Eight had a history of rheumatic diseases, 7 of syphilis, 5 were chronic alcoholics, 9 had brain diseases including 4 with epilepsy

Conclusion

It is evident from this review of the complications and causes of death in polycythaemia vera that without treatment the prognosis is poor In looking through all the case records we found that patients who stopped their follow up visits for some reason or other again developed severe complications, although previously their blood values had been rendered normal by treatment. Accordingly the patients ought to be seen at least several times annually so that there is a chance of intervening if their blood values should change or their symptoms get worse Patients on chlornaphazine therapy have to be seen at short intervals, especially during the initial phase of the treatment while large doses are being administered

Venesection may be used to initiate treatment with mitostatics, until these drugs can be expected to act upon the bone marrow but should be avoided as continued treatment and reserved for cases requiring quick relief.

X ray therapy whose efficacy is apparent from the figures, was cumbersome and fatiguing for the patients. The common occurrence of myelofibrosis or leukaemia was mentioned above. Generalized X radiation appears to have been most effective while irradiation of the pylorus was ineffective Irradiation of the spleen had a favourable effect upon the splenic pain. P^{32} therapy is now preferable to X ray therapy which should be reserved for the few cases in which P^{32} or chlornaphazine fail or have to be abandoned because of the side effects. P^{32} alone does not appear to be advisable if the total dose has to be high as this entails a risk of myelofibrosis or leukaemia.

After treatment with chlornaphazine in large doses for some length of time 3 out of 26 patients developed cancer of the urinary tract.

In planning the treatment of polycythaemia due regard has to be paid to the serious side effects. The most reasonable method appears to be P^{32} alternating with chlornaphazine as this can keep the dosage of both at a low level. The patients should be followed up for the rest of their lives.

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was followed by severe gastritis. One patient developed haematemesis and one melaena on chlornaphazine therapy In two cases the treatment had to be aban doned because of bleeding tendency

Other complications

Gout was a common complication, oc curring in 33 cases (13 %) 27 males and 6 females All had elevated values of serum uric acid Several of these patients were totally disabled by this complica cation, having chronic polyarthritis with frequent exacerbations.

Gastric or duodenal ulcer Dyspepsia was a common incipient symptom (cf. table II) A total of 54 patients (22 %) had dyspeptic complaints, 38 males and 16 females. Of these 35 showed X ray evi dence of peptic ulcer (25 males and 10 females) but X ray examination had not been carried out in all cases.

The incidence of peptic ulcer was higher in this series than in the general adult population (14 % as compared with 7 %)

Other diseases

Severe obesity was present in 15 cases, diabetes mellitus in 10 Seven patients had goitre, including one with thyrotoxicosis Eight had a history of rheumatic diseases 7 of syphilis, 5 were chronic alcoholics, 9 had brain diseases including 4 with epilepsy

Conclusion

It is evident from this review of the complications and causes of death in polycythaemia vera that without treat ment the prognosis is poor In looking through all the case records we found that patients who stopped their follow up visits for some reason or other again developed severe complications, although previously their blood values had been rendered normal by treatment. Accordingly the patients ought to be seen at least several times annually so that there is a chance of intervening if their blood values should change or their symptoms get worse. Patients on chlornaphazine therapy have to be seen at short intervals, especially during the initial phase of the treatment while large doses are being administered.

Venesection may be used to initiate treatment with mitostatics, until these drugs can be expected to act upon the bone marrow but should be avoided as continued treatment and reserved for *cases requiring quick relief*

X ray therapy whose efficacy is apparent from the figures, was cumbersome and fatiguing for the patients. The common occurrence of myelofibrosis or leukaemia was mentioned above. Generalized X radiation appears to have been most effective, while irradiation of the pylorus was ineffective. Irradiation of the spleen had a favourable effect upon the splenic pain. P^{32} therapy is now preferable to X ray therapy which should be reserved for the few cases in which P^{32} or chlor naphazine fail or have to be abandoned because of the side effects. P^{32} alone does not appear to be advisable, if the total dose has to be high, as this entails a risk of myelofibrosis or leukaemia.

After treatment with chlornaphazine in large doses for some length of time 3 out of 26 patients developed cancer of the urinary tract.

In planning the treatment of poly cythaemia due regard has to be paid to the serious side effects The most reasonable method appears to be P^{32} alternating with chlornaphazine as this can keep the dosage of both at a low level. The patients should be followed up for the rest of their lives

Summary

The authors review a series of 250 patients with polycythaemia vera from 1933—1961 The course of the disease was elucidated by looking through the case records from all admissions to hospital.

The causes of death are analysed Forty per cent died of thrombosis, occurring in a total of 158 patients (63 %) Cerebral thrombosis was most common. Haemorrhage was less common. A total of 14 patients developed myelofibrosis, 9 leukaemia, and 6 patients other forms of anaemia.

The results of the various methods of treatment are compared. Out of the untreated patients 50 % had died 18 months after the onset of the first symptom or sign, and out of patients treated with venesection half had died 3 ½ years after this juncture. Out of patients treated by X-rays half were alive 12 ½ years after the first sign. As yet, it is difficult to evaluate the treatment with P³² and chlornaphazine, because the observation period is still short.

The differential diagnostic difficulties in dealing with leukaemia myelofibrosis-anaemia are mentioned. The role of treatment in the development of myelofibrosis and leukaemia is pointed out. Out of 7 patients treated with large doses of X-rays as well as P³² 5 developed myelofibrosis or leukaemia.

Out of 26 patients treated with large doses of chlornaphazine 3 developed cancer of the urinary tract.

Out of other complications, gout, nephrolithiasis, and gastric or duodenal ulcer are mentioned.

In order to reduce the number of side effects, it is recommended to combine P³² with periods of chlornaphazine and to follow the patients carefully throughout life.

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In planning the treatment of polycythaemia due regard has to be paid to the serious side effects. The most reasonable method appears to be P^{32} alternating with chlornaphazine as this can keep the dosage of both at a low level. The patients should be followed up for the rest of their lives.

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Shock after Administration of Phenothiazines in Patients with Pheochromocytoma

By

Per Lund-Johansen

Pheochromocytoma is such a rare disease that the discovery of two typical cases in one practice in one year was published recently (15) The diagnosis is often very difficult, both when the symptoms appear in paroxysms and when the disease manifests itself by permanent hypertension.

The purpose of this paper is to report an unusual case of pheochromocytoma and to draw attention to a little known reaction in pheochromocytoma-patients — a pronounced sensitivity to the hypotensive effect of the phenothiazines. The knowledge of this reaction might be useful in establishing the diagnosis.

Case report

A 51-year-old farmer became acutely ill on April 17 1961 with severe abdominal pain, nausea, vomiting, fever pollakiuria and penile pain. After 3 days he was admitted to the surgical department of this hospital. On admission he was very weak, dehydrated, semicomatous, and there was a peculiar colour of the skin. The face, neck and palms were carrot-red, while the body and the rest of the extremities were pale with cyanotic and red spots (fig. 1) He was cold, sweating and the rectal temperature 40.2° C, the pulse 120 regular and weak. In great contrast to his appearance of shock, the B.P was 220/120. Th abdomen was moderately distended and tender The ocular fundi were normal. The physical examination disclosed no further abnormalities.

Laboratory findings. Haemoglobin level 124% W.B.C. 15,000/mm³ blood sugar level 304 mg % The urine contained albumin and glucose, no pus or blood. ECG showed left ventricular hypertrophy

Ten years earlier the patient had been in hospital, with *identical* symptoms and findings, and the B.P on admission was then 215/100. He was treated with antibiotics and fluids, and was afebrile after 5 days. He left th hospital after 12 days, apparently well, with normal blood pressure.

As for the *intercurrent 10 years* we were first informed that the patient had been quite well, but later it was evident that in the last 2 years he had suffered from attacks of abdominal pain, during which he turned red in the face and hands. During the attacks, which usually lasted one day he often went to bed, but did not call the doctor (He was unmarried.) H never complained of headache.

One hour after admission in 1961 he was moribund, rectal temperature 41.5 C. He was given infusions of saline and glucose, and antibiotics. Hypothermal treatment was insti-

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Fig. 1. The patient on admission.

Table I Results of biochemical analyses

Date (April 1961)	20	21	22	23	24	25	26	27	28
Diuresis (ml/24 hrs)	+225	620	1,900	2,200	4 400	2,100	900	200	0
Haemoglobin (%)	124	125	107	—	99	—	—	—	—
Urea (serum, mg %)	75	210	330	—	—	280	—	—	—
Glucose (serum, mg %)	304	274	199	—	192	—	—	—	—
Adrenalin (urine, μg/24 hrs)	1,900	1 070	—	—	4 100	—	—	—	—
Noradrenalin (urine, μg/24 hrs)	1,680	1,050	—	—	9 600	—	—	—	—

tuted, and *50 mg chlorpromazine* given intramuscularly During the following hour the B.P fell from *210/120* to *80/50* and he had the clinical appearance of shock. Pressor infusion was made ready but the blood pressure then rose spontaneously to 120/100 The following two days it was around 150 systolic.

The diagnosis of pheochromocytoma, carcinoid syndrome and acute pancreatitis was discussed the last soon eliminated. In the present condition the patient was not fit for operation. We hoped that he would recover from the acute condition as he did 10 years ago, and later could be submitted to the tests necessary to diagnose and localize the tumour Urine was immediately sent to laboratory for adrenalin-noradrenalin and 5-HIIA analyses. Screening tests for 5-HIIA was negative.

After two days the patient was better but still had uremia. The blood pressure then rose (fig 2) and the patient developed permanent urinary retention and pronounced meteorism The flushing lasted for 5 days. He died in a cachectic condition with anuria and uremia after 8 days.

From fig 2 it is seen that after the injections of 12.5 mg chlorpromazine given every 6th hour (as a part of the hypothermal treatment) the B.P usually drops moderately and the effect is less marked after 3—4 days. On the 4th day because of hypertensive crisis and hyperpyrexia, *25 mg chlorprothixen (Truxal)* was given intramuscularly The B.P fell from 210/140 to *130/100*

At autopsy we found the left adrenal gland hypertrophic, and in the right was a tumour the size of a tangerine with necrotic and haemorrhagic areas. The histological diagnosis was bilateral pheochromocytoma. The heart showed left ventricular hypertrophy

The results of the biochemical analyses were present after the patient's death. They showed excessive amounts of both adrenalin and noradrenalin (table I)

Comments

It is most probable that the patient referred to suffered from undiagnosed pheochromocytoma already in 1951 The description of the symptoms and findings was identical with those recorded in 1961 when the diagnosis was verified at autopsy It is remarkable that he recovered spontaneously from the attack during which he was moribund, and for the following 8 years was nearly symptom free. It has been supposed by Brody 1959 (2) that when the production of noradrenalin is very high it will cause so much local vasoconstriction that parts of the tumour will become necrotic. It is probable that this happened to our patient 10 years ago.

The long duration of the flush is unusual. Kennedy 1961 (10) however reported a 35-year-old patient with no previous symptoms other than a confluent erythema present for some months, who suddenly developed a crisis with abdominal pain, vomiting dysuria and hyperpyrexia and died. The autopsy showed bilateral large necrotic pheochromocytomata.

Urinary retention a prominent symptom in our patient, is seldom reported in

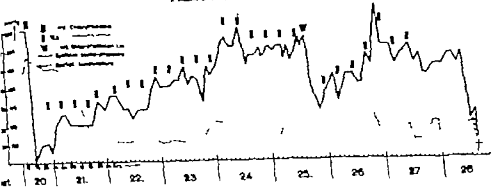


Fig. 2. The patient' blood pressure and rectal temperature.

patients with pheochromocytoma, and is due to contraction of the internal sphincter and relaxation of the detrusor muscles, caused by noradrenalin (1)

The differential diagnosis between pheochromocytoma and carcinoid syndrome is seldom discussed, but might be very difficult as in our case, especially if abdominal pain, meteorism and flushing are prominent. Fluctuations in the blood pressure might be seen in the carcinoid syndrome (9) However *hyperpyrexia*, a usual finding during attacks in pheochromocytoma patients (on Euler and Ström (5) present when recorded in 20 of 27 cases in the large analysis by Graham (8) is not reported in large series or reviews on the carcinoid syndrome (4 14 17) Before the diagnosis can be settled by examination of the catecholamines in the urine, the easy screening test for indols (positive in the carcinoid syndrome) might be useful, but the reaction is not specific and might be useless if the patient has been given phenothiazine (14)

The blood pressure. A moderate fall in the blood pressure after administration of phenothiazine is well known. It is shown experimentally that they act as antagonists on the pressor effect of noradrenalin (6 However a massive fall in the blood pressure leading to circulatory collapse after a small dose of these drugs, is unusual. It might be observed during anaphylactic reactions, but the response later excluded this in our patient. Nor are hypertensive individuals more sensitive to the hypotensive effect of the phenothiazine (16) A similar reaction after the administration of phenothiazine during hypertensive crises with abdominal pain and vomiting in patients with pheochromocytoma has been published. Lima et al. (11) reported sudden shock after 25 mg *chlorpromazine* intramuscularly with fall in the blood pressure from 280/160 to 70/30 Later oral doses made the hypertensive crises less frequent and intense. A patient reported by Brody (2) was given 10 mg *prochlorperazine* intramuscularly and the blood pressure rapidly fell from 190/150 to 80/60 and levarterenol had to be given. Later the patient was normotensive for 3 weeks. Brody suggests that the sudden development of shock might have produced circulatory disturbances and necrosis in the tumour making this inactive. After this episode, there was a decline to normal of the urinary catecholamine levels. In our patient the tumour continued secreting large quantities of adrenalin and noradrenalin. In our case as well as in Lima s, the chock reaction

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followed the first dose of phenothiazine. Later doses had only a moderate hypotensive effect. The two observations reported together with ours, demonstrate clearly that both chlorpromazine, prochlorperazine and chlorprothixen might give sudden hypotension in pheochromocytoma patients. However spontaneously occurring hypotension in these patients has been described (1 12) and explained by the finding of tumours secreting mainly adrenalin (12) which is a general vasodilatator not giving hypertension (7) It is possible that other drugs than the phenothiazines might give similar reactions — unexpected shock during anaesthesia has been reported (13) It is well known that the Regitine test might result in shock, and pressor injections should be ready

Many of the symptoms occurring in pheochromocytoma patients, from slight hypertension with nervousness and restlessness to severe attacks of abdominal pain, vomiting and hyperpyrexia might lead the physician to administer a drug from the phenothiazine group. If the blood pressure falls remarkably after a small dose, pheochromocytoma should be suspected If the patient's condition does not permit of the necessary diagnostic tests in order to localize the tumour hesitation in operating might be dangerous. Upper transverse incision should be performed since the tumour is intra abdominal in 95 % of the cases (3)

Summary

A patient with pheochromocytoma recovered spontaneously from a life threatening hypertensive crisis and was free from symptoms for 8 years. After 10 years he developed a new and severe crisis, with abdominal pain, vomiting flushing and urinary retention as prominent symptoms. The administration of chlorpromazine and later chlorprothixen was followed by a sudden and massive fall in the blood pressure. The significance of these reactions is discussed. The differential diagnosis between pheochromocytoma and carcinoid syndrome is briefly discussed It is stressed that unexpected circulatory collapse after small doses of the phenothiazines should raise a strong suspicion of the presence of pheochromocytoma.

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From the First Medical University Clinic (Chief: C. Holten, M. D.)
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Spontaneous Rupture of a Papillary Muscle of the Heart

Report of three Cases

By

JACOB HANSEN

Rupture of papillary muscles of the heart is a diagnosis which is usually made only at autopsy. However, in 1948 Davison (4) made the first correct antemortem diagnosis, and collected 29 cases from the literature. In 1950 Askey (1) published eight additional cases, including two diagnosed before death, and Schwartz and Canelli (9) reported a case also diagnosed during life. In 1953 Craddock and Mahe (3) reported one case and reviewed 43 from the literature. Since then, several additional cases of rupture of papillary muscles of the heart have been described by English, Finnish, German and American authors (2, 5, 6, 7, 10, 11, 12). In 1957 Sanders et al. (8) published several cases of rupture of papillary muscles and reviewed the aetiology of the 61 cases so far on record. The causes of the rupture were: myocardial infarction, 49 cases, trauma, 6, syphilis 2, endocarditis, 2, polyarteritis nodosa, 1, while the causative factor was unknown in one case.

Among the 43 cases considered by Craddock and Mahe (3) the rupture involved the posterior papillary muscle of the left ventricle in 27 cases and the anterior in 10, while rupture of the papillary muscle of the right ventricle had occurred only in two cases. The site of the rupture was not reported in four cases.

Diagnosis, differential diagnosis and clinical course

In most cases on record the clinical picture was characterized by intense retrosternal pain followed by sudden shock and pulmonary oedema leading to death within a few hours or days. It has been reported (3) that a systolic murmur at the apex, which had not previously been present, developed in 50 % of the cases. Obviously it will often be difficult to hear this murmur if pronounced pulmonary oedema is present. It must, however, be emphasized that rupture of a papillary muscle or of the interventricular septum must be suspected in patients in whom a loud systolic murmur suddenly appears.

By auscultation alone it is very difficult to differentiate rupture of a papillary

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120- weak systolic murmur was heard at the apex; blood pressure 75/50.

Moist rales were audible over the lungs. The ECG showed definite signs of coronary occlusion with pattern of posterior-wall infarction. Prothrombin index 79 (Quick) Hb 93 %.

Anticoagulant therapy with heparin prolongatum and dicumarol was instituted, and noradrenaline infusion was given to combat the shock, but 4 hours after admission sudden death occurred.

Autopsy Both lungs were very oedematous. The right coronary artery was completely occluded by thrombotic masses, and fresh myomalacia of the posterior wall of the left ventricle was present with rupture of the posterior papillary muscle; the anterior part of the muscle had been torn off and was hanging freely in the chordae tendineae attached to the aortic cusp of the mitral valve.



Fig 1 Case 3. Two free ends of the partially ruptured papillary muscle are seen in the upper part.

Case 3. A man, aged 63, had been admitted to the medical department with peptic ulcer 13 years previously No dyspeptic symptoms had been present in the meantime. Six years ago he had been admitted to the surgical department with a pelvic fracture. Two days before the present admission he had an attack of mild retrosternal pain, and on the day of admission protracted attack of very intense pain in the chest developed, the pain radiating to the neck and right arm. On admission the patient was greatly distressed, but not in shock.

Auscultation revealed rapid heart rate, but no murmurs. Blood pressure 130/100 Electrocardiography showed large infarct of the anterior wall. The serum glutamic acid-oxaloacetic acid transaminase concentration was about 165 units (normal value below 40 units). Prothrombin index 93 (Quick) The patient was treated with heparin prolongatum and dicumarol in addition, analgesics often had to be given in order to relieve recurrent attacks of retrosternal pain. During the first three weeks in hospital, the transaminase level which had decreased after the initial attack on three occasions rose to 700 units. Simultaneously with the last rise the condition showed marked deterioration, shock developed with venous congestion in the neck and Cheyne-Stokes respiration. Blood pressure 100/70. Auscultation revealed signs of intense pulmonary congestion and, at the apex of the heart, weak systolic murmur which had previously not been present. 1 1/2 hours later auscultation was again undertaken by the same doctor The cardiac action was slightly irregular (extrasystoles) and the systolic murmur had changed completely into sound of high frequency maximal in the fourth intercostal space midway between the left sternal margin and the mid clavicular line. The following note was made in the record Rupture of papillary muscle suspected. Death ensued 15 hours later

Autopsy The lungs were large and congested, with gross oedema. Marked atheromatosis of both coronary arteries was noted. At its origin, the left coronary artery was occluded by a fresh thrombus adherent to the wall. The myocardium showed extensive, yellow fairly firm areas of myomalacia involving the entire anterior wall, the inter ventricular septum and part of the posterior wall.

Part of the anterior papillary muscle of the left ventricle had ruptured at its origin from the wall. The ends of the chordae tendineae were rounded, with subendocardial haemorrhages. The entire papillary muscle was necrotic and covered by layer of fibrin (fig. 1)

muscle from that of the interventricular septum. Askey (1) stated that the systolic murmur in ruptured interventricular septum is often accompanied by a systolic thrill while it has been said that thrills are never felt in rupture of a papillary muscle. The very presence, however of a thrill is scarcely a decisive factor in the differential diagnosis since a thrill only signifies a murmur so loud that it makes the thoracic wall vibrate. In ruptured interventricular septum the murmur will usually have its maximum intensity at the sternum while in rupture of a papillary muscle the murmur is most distinctly audible at the apex of the heart.

In the First Medical University Clinic, Aarhus Kommunehospital we have during the last 4 years seen three cases of rupture of a papillary muscle all in patients with myocardial infarction. Since as appears from the above account such cases are relatively rare, it may be of interest to have them published.

Case reports

Case 1 A man, aged 59 had 2 years previously been admitted to another hospital with coronary occlusion. Since then, only moderate cardiac distress had been present 5 1/2 hours before admission he complained of sudden intense retrosternal pain. On admission, the patient was greatly distressed, but not in shock. Auscultation showed a regular normal rhythm and no murmurs. Blood pressure 125/100 Auscultation of the anterior surface of the chest revealed no pulmonary abnormalities. The ECG showed remnants of an old infarction with deep QII and QIII waves and signs of a fresh infarction of the posterior wall. Prothrombin index 85 (Quick) Hb 103 / The patient was treated with heparin prolongatum and dicumarol. Four weeks after admission the treatment was discontinued, and the patient was allowed out of bed, but two days later fever developed accompanied by auscultatory signs of bilateral bronchopneumonia. He was treated with dipenicillin, 400 000 units twice daily following which the temperature returned to normal. One week later intense retrosternal pain suddenly developed, followed by shock with severe venous congestion and a systolic jugular venous pulse. The ECG showed right axis deviation, but no signs of recurrent occlusion. According to the record, pulmonary embolism or a small rupture should be suspected. One hour later the systolic blood pressure fell to 75 mm Hg five hours later electrocardiography showed increasing right axis deviation, and the blood pressure was unmeasurable. Noradrenaline infusion was instituted, but the patient died about 7 hours after the onset of the acute episode.

Autopsy The lungs were slightly oedematous, without signs of infarction. The pulmonary arteries did not contain any emboli. The heart weighed 745 g and showed pronounced generalised myocardial hypertrophy and dilatation of all cavities. In the septum a large area of fibrosis was revealed, but no signs of fresh changes. The posterior wall of the heart contained a very large, fresh infarction. Rupture of the heart or its septum was absent. Both coronary arteries showed pronounced atheromatosis. At a distance of 4 cm from the ostium, the right coronary artery contained a partially organised thrombus, and distal to this, a long typical, subintimal haemorrhage was seen. Both papillary muscles of the left ventricle were necrotic, and the posterior muscle had ruptured.

Histological examination of the ruptured papillary muscle revealed marked basal fibrosis. Most of the muscle was the site of fresh myomalacia. The myocardial cells were completely structureless and without nuclei. At the site of rupture, the tissue was also structureless with infiltration with polymorphonuclear leukocytes, including numerous eosinophils.

Case 2 A woman, aged 79 with a history of good health, felt increasingly tired and dyspnoeic during the four days before admission. Six hours before admission, intense precordial pain suddenly developed On admission the patient was distressed cold and perspiring

Auscultation of the heart revealed distant heart sounds and a regular action pulse rate

120- weak systolic murmur was heard at the apex blood pressure 75/50.

Moist rales were audible over the lungs. The ECG showed definite signs of coronary occlusion with pattern of posterior-wall infarction. Prothrombin index 79 (Quick) Hb 93 %.

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Autopsy. Both lungs were very oedematous. The right coronary artery was completely occluded by thrombotic masses, and fresh myomalacia of the posterior wall of the left ventricle was present with rupture of the posterior papillary muscle the anterior part of the muscle had been torn off and was hanging freely in the chordae tendineae attached to the aortic cusp of the mitral valve.

Case 3. A man, aged 65, had been admitted to the medical department with peptic ulcer 13 years previously No dyspeptic symptoms had been present in the meantime. Six years ago he had been admitted to the surgical department with pelvic fracture. Two days before the present admission he had an attack of mild retrosternal pain, and on the day of admission protracted attack of very intense pain in the chest developed, the pain radiating to the neck and right arm. On admission the patient was greatly distressed, but not in shock.

Auscultation revealed rapid heart rate, but no murmurs. Blood pressure 130/100. Electrocardiography showed large infarct of the anterior wall. The serum glutamic acid-oxaloacetic acid transaminase concentration was about 165 units (normal value below 40 units). Prothrombin index 93 (Quick) The patient was treated with heparin prolongatum and dicumarol in addition, analgesics often had to be given in order to relieve recurrent attacks of retrosternal pain. During the first three weeks in hospital, the transaminase level which had decreased after the initial attack on three occasions rose to 700 units. Simultaneously with the last rise the condition showed marked deterioration, shock developed with venous congestion in the neck and Cheyne Stokes respiration. Blood pressure 100/70. Auscultation revealed



Fig 1 Case 3. Two free ends of the partially ruptured papillary muscle are seen in the upper part.

signs of intense pulmonary congestion and, at the pex of the heart, weak systolic murmur which had previously not been present. 1 1/2 hours later auscultation was again undertaken by the same doctor The cardiac action was slightly irregular (extrasystoles) and the systolic murmur had changed completely into sound of high frequency maximal in the fourth intercostal space midway between the left sternal margin and the midclavicular line. The following note was made in the record Ruptur of papillary muscle suspected. Death ensued 15 hours later

Autopsy The lungs were large and congested, with gross oedema. Marked theromatosis of both coronary arteries was noted. At its origin, the left coronary artery was occluded by fresh thrombus adherent to the wall The myocardium showed extensive, yellow fairly firm areas of myomalacia involving the entire anterior wall, the inter ventricular septum and part of the posterior wall.

Part of the anterior papillary muscle of the lef ventricle had ruptured a its origin from the wall. The ends of the chordae tendineae were rounded, with subendocardial haemor rhages. The entire papillary muscle was necrotic and covered by a layer of fibrin (fig. 1)

muscle from that of the interventricular septum Askey (1) stated that the systolic murmur in ruptured interventricular septum is often accompanied by a systolic thrill, while it has been said that thrills are never felt in rupture of a papillary muscle. The very presence however of a thrill is scarcely a decisive factor in the differential diagnosis, since a thrill only signifies a murmur so loud that it makes the thoracic wall vibrate. In ruptured interventricular septum the murmur will usually have its maximum intensity at the sternum while in rupture of a papillary muscle, the murmur is most distinctly audible at the apex of the heart

In the First Medical University Clinic, Aarhus Kommunehospital, we have during the last 4 years seen three cases of rupture of a papillary muscle, all in tients with myocardial infarction Since as appears from the above account, such cases are relatively rare, it may be of interest to have them published

Case reports

Case 1 A man, aged 59 had 2 years previously been admitted to another hospital with coronary occlusion. Since then, only moderate cardiac distress had been present. 5 1/2 hours before admission he complained of sudden intense retrosternal pain. On admission, the patient was greatly distressed, but not in shock. Auscultation showed a regular normal rhythm and no murmurs. Blood pressure 125/100. Auscultation of the anterior surface of the chest revealed no pulmonary abnormalities. The ECG showed remnants of an old infarction with deep Q_{II} and Q_{III} waves and signs of a fresh infarction of the posterior wall. Prothrombin index 85 (Quick) Hb 103 % The patient was treated with heparin prolongatum and dicumarol. Four weeks after admission the treatment was discontinued, and the patient was allowed out of bed, but two days later fever developed accompanied by auscultatory signs of bilateral bronchopneumonia. He was treated with dipenicillin, 400,000 units twice daily following which the temperature returned to normal. One week later intense retrosternal pain suddenly developed followed by shock with severe venous congestion and a systolic jugular venous pulse. The ECG showed right axis deviation, but no signs of recurrent occlusion. According to the record, pulmonary embolism or a small rupture should be suspected. One hour later the systolic blood pressure fell to 75 mm Hg five hours later electrocardiography showed increasing right axis deviation, and the blood pressure was unmeasurable. Noradrenaline infusion was instituted, but the patient died about 7 hours after the onset of the acute episode.

Autopsy The lungs were slightly oedematous, without signs of infarction. The pulmonary arteries did not contain any emboli. The heart weighed 745 g and showed pronounced generalised myocardial hypertrophy and dilatation of all cavities. In the septum a large area of fibrosis was revealed, but no signs of fresh changes. The posterior wall of the heart contained a very large, fresh infarction. Rupture of the heart or its septum was absent. Both coronary arteries showed pronounced atheromatosis. At a distance of 4 cm from the ostium, the right coronary artery contained a partially organised thrombus, and distal to this, a long typical, subintimal haemorrhage was seen. Both papillary muscles of the left ventricle were necrotic, and the posterior muscle had ruptured.

Histological examination of the ruptured papillary muscle revealed marked basal fibrosis. Most of the muscle was the site of fresh myomalacia. The myocardial cells were completely structureless and without nuclei. At the site of rupture, the tissue was also structureless with infiltration with polymorphonuclear leukocytes, including numerous eosinophils.

Case 2 A woman, aged 79 with a history of good health, felt increasingly tired and dyspnoeic during the four days before admission. Six hours before admission, intense precordial pain suddenly developed. On admission the patient was distressed cold and perspiring

Auscultation of the heart revealed distant heart sounds and a regular action pulse rate

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Urinary Excretion of Histamine in Patients with Dystrophia myotonica

By

Ottar Sjaastad and Ottar Sjaastad

The coexistence of increased urinary excretion of histamine and osteosclerosis has been demonstrated in patients with ventricular carcinoid tumours (10 12). This has led to the assumption of a cause and effect relationship between these two conditions (12).

Localized increase in thickness and density of the bone of the skull, so-called hyperostosis frontalis interna, is frequently encountered in patients with dystrophia myotonica. Consequently a study of histamine metabolism in these patients seemed indicated.

Material and methods

Nine female and seven male patients in hospital suffering from dystrophia myotonica form the basis of this study which was performed between September 1961 and January 1962. The patients have been classified in four groups, for rough estimation of their general clinical condition:

+ Patients having no disability for work.

++ Patients partly incapacitated for work.

+++ Patients almost or completely incapacitated for work, but ambulatory.

++++ Bedridden patients.

According to this categorization the patients in this study belong to groups + to +++ (table II).

Two groups, viz. six patients in hospital, mostly psychoneurotics, and nine healthy students were studied for control of urinary content of histamine. None of the patients suffering from dystrophia myotonica received quinine. Some of them, however received various other drugs during the study (table II).

For histamine determination, twenty-four hour urine samples were collected from patients and controls. The extraction of histamine was performed as outlined by Dunér and Pernow (5) using an Amberlite IRC-50 column for adsorption of histamine. The acetyl-conjugated histamine was determined as the increase in free histamine which occurred upon acid hydrolysis. The hydrolysis was performed by boiling the urine on a hot plate for 1 1/2 hours, using 10 N hydrochloric acid.

Histamine was assayed conventionally on isolated atropinized guinea-pig ileum, and the values presented are calculated as histamine base.

Skull thickness has been measured as described by Oldberg (8).

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Comments

The symptomatology of these cases is in close agreement with the descriptions reported in the literature. The predominant symptoms are (1) Intense pain resembling that of acute myocardial infarction, (2) shock and (3) pulmonary oedema. The sudden appearance of a systolic murmur at the apex or a little to the right of the apex is strongly suggestive of rupture of a papillary muscle or of the interventricular septum.

In all probability rupture of a papillary muscle in patients with myocardial infarction most frequently occurs within a few hours after the development of the infarction. This assumption is supported by cases reported by Craddock and Mahe (3) Segall and Sharp (10) and Askey (1)

In case 1 reported above myocardial infarction supervened on the 5-week-old posterior wall infarction, and it must be assumed that this development was immediately followed by rupture of the papillary muscle, since autopsy showed that this muscle was the site of recent myomalacia

In case 2 it is probable that the infarction developed about 6 hours before admission, and that rupture occurred shortly afterwards since the patient died about 10 hours after the onset of pain.

Judging from the transaminase determinations repeated extensions of the infarction occurred in case 3 A loud systolic murmur was audible 1 1/2 hours after the patient had passed into shock. The rupture probably occurred during this period

Summary

A brief review of important publications on rupture of papillary muscles is presented The cause of such rupture is in most cases myocardial infarction, and the clinical picture is usually characterized by sudden shock and pulmonary oedema. An ante-mortem diagnosis has been made only in exceptional cases. Rupture of a papillary muscle should be suspected on the sudden appearance of a loud systolic murmur at the apex in patients with myocardial infarction, but the differential diagnosis from rupture of the interventricular septum is difficult.

Three cases, all with myocardial infarction are reported. The rupture involved the posterior papillary muscle in two and the anterior in one. In one case, a loud systolic murmur at the apex suddenly appeared so that the diagnosis of rupture of a papillary muscle was made during life. In another case, rupture was strongly suspected. Autopsy confirmed this suspicion.

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Table II. Urinary histamine excretion, skull thickness and clinical data in 16 patients with dystrophia myotonica

Case	Sex	Age	Clinical condition	Skull thickness in mm		Histamine values in µg/24 hours		Drugs **
				F*	P*	Free	Conjugated	
1	F	54	+	10.5	10.8	2.5	3,633	0
2	F	40	++	11.0	9.8	34.3	1,275	0
3a	F	43	+++	13.3	12.3	9.9 }	938 }	Meprobamate
3b						4.5 }	889 }	Salicylic acid
4	M	48	++	9.8	9.8	3.6	850	0
5	M	49	+++	4.7	7.8	6.6	244	Barbiturates Atropine
6	M	41	++	12.5	12.0	5.9	201	Barbiturates Salicylic acid
7	F	56	+++	9.5	7.3	15.8	121	0
8	M	39	+++	7.8	7.8	0.3	105	0
9	F	40	++	14.0	12.0	90.2	23.4	0
10a	F	40	+	11.3	10.3	67.5 }	87.1 }	0
10b						86.8 }	43.9 }	
11	F	21	+	7.8	9.5	17.7	25.8	Barbiturates Salicylic acid
12	M	48	+++	11.8	8.8	18	18.3	0
13	F	54	+	9.5	9.0	16.8	14.5	Phenacetin Barbiturates
14	F	58	+++	6.8	6.7	15.2	11.9	0
15	M	14	+	5.3	7.3	18.4	12.1	0
16	M	50	+++	9.5	10.5	8.5	1.3	0

Classification + to ++++ see Material and methods. P* = frontal thickness. F* = parietal thickness. Drugs*** = drugs given before or during collection of urine.

In patients with normal or moderately increased elimination of histamine there was no definite correlation between histamine levels and skull thickness.

Discussion

Increased urinary output of histamine has previously been reported in a few pathological conditions, e.g. small intestinal (9) and ventricular carcinoid tumours (12), urticaria pigmentosa (mastocytosis) (4) and basophile leukemia (3). In these pathological conditions both free and conjugated urinary histamine levels were elevated.

The present study demonstrates the presence of elevated urinary histamine levels in patients with dystrophia myotonica. To the best of our knowledge, solitary or predominant elevation of conjugated histamine, as in our patients has not been reported previously.

According to Adam (1) orally administered histamine is almost entirely excreted as the conjugate. Contrarily parenteral application of histamine results in increased excretion essentially of free but also of conjugated histamine (6 11). This may indicate that the source of elevated urinary histamine excretion in patients with dystrophia myotonica is the alimentary tract. None of the pa

Table I Urinary excretion of free and conjugated histamine in a control group consisting of 9 healthy students and 6 psychoneurotic patients from the same ward as the patients with dystrophia myotonica

No.	Sex	Age	Histamine values in µg/24 hours	
			Free	Conjugated
Students				
1	M	28	10.2	9.5
2	M	24	20.2	10.0
3	F	22	5.6	22.5
4	F	24	14 1	8.7
5	M	21	5.0	17.5
6	M	20	1.8	4.9
7	M	20	5.6	16.6
8	M	22	13.0	13 6
9	M	34	15.6	52 4
Hospitalized patients				
10	M	41	8.5	72.0
11	F	42	7.3	13.0
12	F	26	25.2	20.0
13	M	51	5.0	71 4
14	M	51	6.6	13.9
15	F	36	4.0	5.4

Evidence that the substance measured is histamine

The effect of the extract on the guinea pig ileum was completely counteracted by small amounts of antihistamine, whereas atropine and dihydroergotamine was without influence. Boiling at high pH destroyed the activity of the extract. Paper chromatography of butanol extracted urine from patient no. 2, using Whatman paper no. I and ethanol/diethyl-ether/distilled water/conc. ammonia 4/5/1/0 1 as moving phase showed an accentuated spot with Rf as N-acetylhistamine standard (Claus Huth) After acid hydrolysis the corresponding spot decreased and a spot occurred at the same level as histamine phosphate standard (L. Light & Co. Ltd) The area of expected histamine on an unstained chromatogram was eluated in Tyrode's solution and tested for activity on the guinea-pig ileum. The eluate showed a definite contracting effect which was inhibited by antihistamine. The remainder of the strip gave an inactive eluate.

Results

The average 24 hour urinary excretion of free histamine in the control group was 9 8 ± 1 7 µg (S.E.M.)[1] whereas the mean excretion of conjugated histamine was 23 4 ± 5 8 µg (see table I) These values are in fairly good agreement with the findings of Dunér and Pernow in 17 normal individuals (5) The average excretion of free histamine was the same in the two control groups, whereas the excretion of conjugated histamine was 17.3 µg in the student group against 32 6 µg in the ward groups.

The respective averages of free and conjugated urinary histamine in µg per 24 hours in 16 patients with dystrophia myotonica were 20 3 ± 6 1 (S E.M) and 473 6 ± 73 9 (see table II)

In ten patients urinary output of free and/or conjugated histamine was above the control range. Three patients showed elevated excretion of free histamine, and one of these three patients of free histamine exclusively (see table II) The correlation between the amount of histamine in urine and the clinical condition was poor (see table II)

Getz (7) has determined the average cranial thickness in the frontal and parietal regions in a group of Norwegians of comparable age employing Oldberg's (8) technique. The average frontal thickness was 6 8 ± 0.2 mm (S.E.M.) and the parietal thickness 7 8 ± 0.2 mm.

The measurements of skull thickness in our material are presented in table II The respective average frontal and parietal thicknesses were 9 7 mm and 9.5 mm. In four patients with markedly increased excretion of histamine, i e. > 800 µg/24 hours, the mean frontal and parietal thicknesses were 11 2 mm and 10 7 mm.

[1] S E.M. = standard error of the mean.

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Specific Muscle Lesions in Pituitary-thyroid Disorders

By

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In 1952, Asboe Hansen et al. (4) described certain lesions of the skeletal muscles of patients suffering from progressive exophthalmos, thyrotoxicosis, acromegaly and myxoedema. These lesions were demonstrated in muscle biopsies fixed in a 4 % solution of lead subacetate and stained with a 1/2 % aqueous solution of toluidine blue or the P.A.S. stain after Hotchkiss. In transversely cut sections they appear as half moon-shaped homogenous masses, adherent to the muscle fibres and on the outside enclosed by a membrane, most probably the sarcolemma. In longitudinal sections these phenomena are fusiform. By electron microscopic studies it was confirmed that these amorphous masses are situated inside the sarcolemma and outside the muscle substance (10). They contain acid mucopolysaccharides, presumably mostly hyaluronic acid. Apart from these "half moons" the interfibrillar connective tissue was found to take a fairly strong metachromatic stain, and the number of mast cells was increased. Since the lesions described were present in skeletal muscles, it is a question of a widespread possibly generalized, muscular disorder. Later Wegelius et al. (15) found similar lesions in the extraocular muscles in three patients suffering from malignant exophthalmos. The accumulation of acid mucopolysaccharides in the connective tissue of the ocular muscles together with the "half moons" may explain the increase in volume of the extraocular muscles in malignant exophthalmos, and may be the crucial cause of the protrusion of the eyeballs (15).

Author's investigations

In the following paper the microscopic changes in the skeletal muscles from 98 patients with pituitary-thyroid disorders are described. Of these patients, 13 had thyrotrophic exophthalmos, 29 exophthalmic goitre, 32 hyperthyroidism without exophthalmos, 5 non-toxic goitre, 10 myxoedema, and 9 acromegaly. In the present series, the cases reported previously are included (4, 15). The muscle biopsies were obtained from the quadriceps of the femur or the biceps of the arm. A few were taken from the temporal muscle and the extraocular muscles. The operations were performed under Vinydan-anaesthesia in order to avoid local effects of the anaesthetic. All biopsies were fixed in a

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tients had experienced episodes of flushing This suggests that release of free histamine into the blood never reaches high levels in these patients.

It has been shown that intake of large amounts of meat leads to considerable increase in excretion of conjugated histamine (2) There is no evidence that the food should occasion the increase in urinary excretion of histamine in our patients. They received the same kind of food as the control ward patients, who had low excretion of histamine However the possibility that nutritional factors have had *some* influence on the results in this material cannot be excluded since there is a moderate difference between the student group and the control ward group Neither can the findings be explained as caused by the administered drugs.

This study offers no definite evidence as to the histamine/skull thickness relationship in patients with dystrophia myotonica It is noteworthy that the technique used implies measurement of skull thickness on the lateral skull roentenogram only Consequently the values are not necessarily the exact expression of the degree of hyperostosis in localized forms.

As some patients with advanced disease had normal excretion of histamine, histamine is probably not the causal factor in dystrophia myotonica. It is possible that other metabolic endproducts of histamine not measurable with this technique, may prevail in the patients with normal excretion of acetylhistamine The question of periodic variations in histamine elimination also remains open. Serial observations of histamine excretion in those patients would therefore be pertinent

Summary

Urinary excretion of free and conjugated histamine respectively in 15 control individuals averaged 9.8 ± 1.7 μg and 23.4 ± 5.8 μg The corresponding values in 16 patients suffering from dystrophia myotonica were 20.3 ± 6.1 and 473.6 ± 73.9 Ten patients with dystrophia myotonica had histamine levels above the control range. Solitary elevation of acetyl conjugated histamine as in most of these patients, has not previously been reported

The correlation between the degree of the disease and urinary excretion of histamine was poor while there was some correlation between skull thickness and histamine excretion

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Furthermore, "half-moons" were found in a diagnostic biopsy from a patient, who clinically had no exophthalmos or other signs of pituitary or thyroid disorder. Later on the death of the patient from chronic pyelonephritis, microscopy of the thyroid gland showed slight degree of colloid goitre.

Finally "half-moons" were found in a patient who underwent partial hypophysectomy because of chromophobe pituitary adenoma. Skin biopsy showed localized myxoedema.

Table I. Appearance of acid mucopolysaccharides (A.M.P.) in the skeletal muscles of 98 patients with pituitary-thyroid disorders

	No. of patients	A.M.P. in muscles
Thyrotrophic exophthalmos		
Hyperthyroid	8	7
Euthyroid.	4	3
Hypothyroid	1	1
Exophthalmic goitre		
Untreated	25	9
Thyroidectomized.	4	3
Hyperthyroidism without exophthalmos		
Untreated	30	13
Thyroidectomized	2	2
Non-toxic goitre	3	2
Myxoedema	10	6
Acromegaly	9	8

Controls

Muscular biopsies from 50 patients without pituitary-thyroid disorders were used as controls. These patients had undergone surgery because of intrathoracic diseases, lumbar disc herniation or inguinal hernia. The muscular biopsies were obtained from the region in which the operation in question had been performed. In none of these cases could "half-moon" formation be demonstrated.

Discussion

An increased activity of the thyrotrophic hormone has previously been demonstrated in progressive exophthalmos, localized myxoedema, hypothyroidism, acromegaly and some cases of thyrotoxicosis. This activity is supposed to play an appreciable part in the pathogenesis of these disorders (cf. Asboe-Hansen (1)).

The thyrotrophic hormone of the anterior pituitary gland is thought to consist of two factors, — a thyroid-stimulating factor and factor stimulating connective tissue (lipotrophic, mucotrophic and ophthalmotrophic) (1). Other authors (6, 7, 8, 11) have succeeded in separating a thyroid-stimulating from an ophthalmotrophic factor. The stimulating effect on the connective tissue may be produced in radically thyroidectomized animals (3, 7). Both factors are inhibited by thyroxin (13).

In some cases of thyrotoxicosis considerable activity of the thyroid-stimulating factor is found. The fact that exophthalmos only develops in some cases of thyrotoxicosis may be due to local conditions of the retrobulbar tissues, e. g. differences in sensitivity to the effect of the ophthalmotrophic factor (2). The fact that the muscle lesions described above are found both in patients with thyrotrophic exophthalmos and with exophthalmic goitre has been interpreted as an indication that different types of endocrine exophthalmos are caused by a common hormonal factor (9).

In "thyrotrophic exophthalmos" the ophthalmotrophic factor predominates. The changes in the retrobulbar connective tissue are identical with the changes which may be produced experimentally in guinea pigs by thyrotrophin stimulation, i. e. oedema of the connec



Fig 1 Microphotograph. Transverse section of muscle fibres from a patient with thyrotrophic exophthalmos. Staining toluidine blue, 1/2 % aqueous solution Note metachromatic half-moons between sarcolemma and muscle fibres, with peripheral nuclei located at the inside of the "half-moons

4 % lead sub-acetate solution for 24 hours and stained by the P.A.S method for polysaccharides and with a 1/2 % aqueous solution of toluidine blue, which rather selectively stains acid mucopolysaccharides metachromatically

Thyrotrophic exophthalmos

In this paper thyrotrophic exophthalmos" means the type of exophthalmos which is supposed to be produced by stimulation of the thyrotrophic hormone. In most cases it develops subsequent to subtotal thyroidectomy In some cases it may be accompanied by localized pretibial myxoedema. In 11 out of 13 patients of this group, intra- or sub-sarcolemmal deposits of acid mucopolysaccharides were demonstrated. In most cases the changes appeared as half-moon like formations in transversely cut sections. In the present material all the patients had undergone antithyroid therapy and half moons" were found both in euthyroid hypo- and hyperthyroid patients. In addition sarcolemmal thickening was demonstrated An increased number of mast cells was found both perivascularly and interstitially and th connective tissue was oedematous and more or less metachromatically stained. In a few specimens, the muscle fibres showed signs of degeneration as blurring of the cross-striation, varying diameters and relative increase in the number of nuclei, but the latter changes were not constant.

Exophthalmic goitre

This group comprises 29 patients, 25 of whom were untreated. Four of the patients had previously been thyroidectomized, but the symptoms had recurred. Half-moons were demonstrated in 12 biopsies, the formations showing the same characteristics and localization as in the group of thyrotrophic exophthalmos.

Hyperthyroidism without exophthalmos

This group comprises 30 patients with goitre and increased basal metabolic rate, but without ocular symptoms. Half-moons were found in 13 patients, independent of the degree of the goitre and the hyperthyroidism. Half moons" were also demonstrated in 2 patients who had previously been thyroidectomized, but in whom the thyrotoxicosis had recurred.

Non-toxic goitre

This group consists of 5 patients, 2 of whom had "half moons" in their skeletal muscles.

Myxoedema

Muscle biopsies were performed in 10 patients with myxoedema. In 6 of these cases "half moons" were found. The number of mast cells was increased and in addition oedema was found. The intercellular connective tissue took a fairly pronounced metachromatic stain.

Acromegaly

In 8 out of 9 patients with acromegaly half-moons" were demonstrated.

Apart from the cases listed in table 1 the skeletal muscles were examined in 7 patients with unilateral monosymptomatic exophthalmos. In 5 of these cases, the exophthalmos was found to be caused by a retrobulbar tumour In none of them could half-moons" be demonstrated. In the last two patients, the exophthalmos disappeared spontaneously after some years. In one of these patients "half-moons" were found in the skeletal muscles.

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tive tissue including the extraocular muscles an increased number of mast cells and an accumulation of acid mucopolysaccharides (7 12 15) Tengroth (14) showed that pepsin-digested thyrotrophin with a major content of the exophthalmos-producing factor induced increased water content both in ocular muscles and in other skeletal muscles in guinea pigs. These observations seem to indicate a general effect of the thyrotrophic hormone.

Following administration of thyroid stimulating hormone to intact and thyroidectomized dogs, Bollet et al. (5) by biochemical analysis, found a quantitative increase in the content of acid mucopolysaccharides in the ocular and skeletal muscles. This effect appears to be direct and not mediated through the thyroid gland

In myxoedema the thyroid gland is affected and thyroid hormone can no longer exert its normal inhibition of thyrotrophin In connective tissue changes are demonstrable that are thought to be brought about by stimulation of the mucotrophic and the lipotrophic factor (4)

Acromegaly is a condition showing increased activity of somatotrophin and thyrotrophin. Increased formation of both mucopolysaccharides and collagen takes place (cf Asboe Hansen (1)

It is possible that the "half moons which have hitherto only been demonstrated in pituitary-thyroid disorders, are produced by an increased thyrotrophin stimulation. However no workers have yet succeeded in producing half moons experimentally Their genesis remains obscure.

Clinically it is often difficult to decide whether a monosymptomatic exophthalmos, especially of the unilateral type, is endocrine or produced by local processes, e. g inflammation or retrobulbar tumour In 11 out of 13 cases of thyrotrophic exophthalmos half moons were found, while no such formations could be demonstrated in 5 patients suffering from retrobulbar tumours nor in 50 control biopsies. Microscopy of skeletal muscles is regarded as an important diagnostic aid in monosymptomatic exophthalmos.

Summary

By means of a special histochemical technique, specific muscle lesions are demonstrated in more than half of 98 patients with pituitary thyroid disorders, i e. in 11 out of 13 patients with thyrotrophic exophthalmos, in 12 out of 29 patients with exophthalmic goitre, in 15 out of 32 patients with hyperthyroidism without exophthalmos, in 2 out of 5 patients with non toxic goitre in 6 out of 10 patients with myxoedema and in 8 out of 9 patients with acromegaly No changes were found in 50 controls and in 5 cases of retrobulbar tumor Hyper activity of the thyrotrophic hormone of the anterior pituitary gland is supposed to play an essential pathogenetic role. Muscle biopsy of skeletal muscles is considered to be of essential diagnostic value in patients with monosymptomatic exophthalmos.

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On Serum Proteins and Erythrocyte Sedimentation Rate in Hypothyroidism

By

Sten Olle Larsson

In 1939 Gildea et al. found that the total serum protein was increased in hypothyroidism (10). It has since been shown experimentally and clinically that impairment of thyroid function may be combined with changes in the serum protein pattern (1, 5, 6, 13, 15, 19, 23, 24, 32, 33). Most workers have reported decrease in the albumin and an increase in the β- and/or γ-globulins, but the results have not always been in accord.

The demonstration of thyroid antibodies in thyroid involvement, especially in Hashimoto disease and hypothyroidism, has aroused renewed interest in the serum protein pattern in affections of the thyroid (4, 7, 8, 9, 11, 14, 25, 26, 27, 28, 31, 35). This paper is concerned with the serum protein pattern and sedimentation rate in untreated idiopathic, postoperative and pituitary hypothyroidism. The cases with idiopathic hypothyroidism, which were divided according to the presence or absence of goitre, were also studied during treatment.

Material and methods

The records of 1954—1960 of the medical department, Malmö General Hospital, were searched for all cases of hypothyroidism. Those cases in which the serum proteins had been determined before treatment had been started and in which the thyroid disease was not complicated by other pathological conditions capable of influencing the serum protein pattern were selected (table I). The 44 patients filling these criteria are therefore not representative of the total number of cases or of the relative frequency of the different types of hypothyroidism seen at the department. For many cases of postoperative hypothyroidism had been treated only at the outpatient department where the serum proteins were not usually assessed and many cases of pituitary hypothyroidism were excluded from the review because of complicating diseases. These two groups were therefore underrepresented.

The diagnosis of hypothyroidism was made from the history, the clinical picture, the basal metabolic rate, the serum protein-bound iodine and the serum cholesterol. The protein-bound iodine was determined by Skanse and Hedenskog's modification of Barker and Humphrey's method (3, 30). The normal mean value for this method is 5.9 with standard deviation of ± 0.7 μg/100 ml. In all cases except the first 4 the serum proteins were studied by paper electrophoresis ac-

Submitted for publication May 7, 1962.

Table I. Distribution of serum protein values in the various groups of hypothyroidism

Aetiology of hypothyroidism	No. of pat.	Serum pro-								
		Total			Alb.			α_1-glob.		
		I	N	D	I	N	D	I	N	D
Idiopatic										
With goitre	10	1	7	2	0	6	4	1	9	0
Without goitre	20	5	13	2	0	16	4	2	16	2
Total	30	6	20	4	0	22	8	3	25	2
Postoperative	9	3	5	1	0	4	5	0	9	0
Pituitary	5	0	4	1	0	4	1	0	5	0
Total	44	9	29	6	0	30	14	3	39	2

I = increased, N = normal, D = decreased.

cording to the method of Laurell et al. (17). According to this method Ca ions are added to the buffer to enable separation of the β-globulins into two fractions of relatively different electrophoretic mobility, the fraction with the higher mobility being called β_1-globulin, the other β_2-globulin. The normal mean values in g/100 ml $\pm$ 2 S.D. are for total protein 7.4 $\pm$ 0.26, albumin 4.7 $\pm$ 0.18, α_1-globulins 0.33 $\pm$ 0.036, α_2-globulins 0.53 $\pm$ 0.05, β_1-globulins 0.54 $\pm$ 0.035, β_2-globulins 0.26 $\pm$ 0.045, γ-globulins 1.06 $\pm$ 0.11. In the first 4 cases the buffer used for electrophoresis did not contain Ca ions: the β-globulins were therefore not separated into two fractions.

Results

Serum protein pattern before institution of treatment with thyroid

It is clear from fig. 1 and table I that the α_1 and α_2-globulin values were with but few exceptions normal in all the groups. This also holds largely for the β_1 globulin values in thyrogenic hypothyroidism while 4 of the 5 patients with pituitary hypothyroidism showed low β globulin values. Otherwise the protein pattern in the cases of pituitary hypothyroidism was largely normal. In thyrogenic hypothyroidism, both postoperative and idiopathic with and without goitre, the albumin value tended to be decreased and, though less markedly in the postoperative group, the β_2-globulin value tended to be increased. Thus, in 13 of the 39 cases of thyrogenic hypothyroidism the albumin was below the lower border of the normal range and in 10 of 35 cases the β_2-globulin value was increased. The most striking finding was, however, the increased concentration of the γ-globulins in idiopathic hypothyroidism, which in this respect differed markedly from the postoperative group. Of the 30 cases of idiopathic hypothyroidism, the γ-globulin concentration was above the upper normal limit in 12. Markedly increased values of more than 1.8 g/100 ml were noted in 4 cases and in one it was as high as 2.23 g/100 ml. No difference in this respect was found between the cases of idiopathic hypothyroidism with goitre and those without.

Table II shows the number of positive T.R.C. tests (tanned-red-cell agglutination) and C.F. tests (complement fixation-capacity) in idiopathic hypothyroid-

tein values

α_2-glob.			β-glob.			γ_1-glob.			γ-glob.		
I	N	D	I	N	D	I	N	D	I	N	D
1	9	0	0	6	3	3	6	0	5	4	1
3	16	1	0	17	0	5	12	0	7	9	4
4	25	1	0	23	3	8	18	0	12	13	5
0	8	1	0	8	1	2	7	0	1	6	2
0	5	0	0	1	4	1	4	0	0	4	1
4	38	2	0	32	8	11	29	0	13	23	8

ism in relation to the γ-globulin concentration. While no statistical difference was found for T.R.C.-tests, cases with increased γ-globulin concentration showed a statistically *higher* frequency of positive C.F.-tests.

No significant correlation was found between the γ-globulin level and the protein-bound iodine in patients with idiopathic hypothyroidism (fig. 2).

One of the patients with idiopathic hypothyroidism with goitre who showed moderately increased γ-globulin concentration was of particular interest because her mother had also had hypothyroidism and hypergammaglobulinaemia. The mother had died from cardiac infarction and postmortem examination had revealed complete atrophy of the thyroid. Table III shows the development of the ESR, BMR, serum cholesterol and serum proteins during thyroid treatment in this case (case M. J.) which is not included in the present material proper because the hypothyroidism had been diagnosed in 1951, i. e. before the

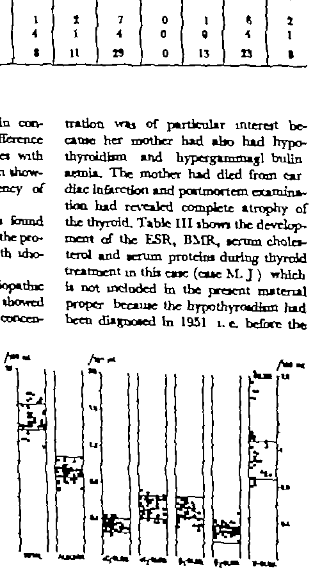


Fig. 1. Serum protein values in all 44 patients studied. The horizontal lines delimit the normal mean ± 2 S. D.

● idiopathic hypothyroidism with goitre.
○ idiopathic hypothyroidism without goitre.
△ postoperative hypothyroidism.
∟ pituitary hypothyroidism.

Table I Distribution of serum protein values in the various groups of hypothyroidism

Aetiology of hypothyroidism	No. of pat.	Serum pro-								
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I = increased, N = normal D = decreased.

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Results

Serum protein pattern before institution of treatment with thyroid

It is clear from fig 1 and table I that the α_1 and α_2-globulin values were with but few exceptions normal in all the groups. This also holds largely for the β_1-globulin values in *thyrogenic hypo*thyroidism, while 4 of the 5 patients with pituitary *hypothyroidism* showed low β globulin values. Otherwise the protein pattern in the cases of pituitary hypothyroidism was largely normal. In thyrogenic hypothyroidism both postoperative and idiopathic with and without goitre, the albumin value tended to be decreased and though less markedly in the post operative group the β_1-globulin value tended to be increased Thus, in 13 of the 39 cases of *thyrogenic* hypothyroidism the albumin was below the lower border of the normal range and in 10 of 35 cases the β_1-globulin value was increased The most striking finding was, however the *increased concentration* of the γ-globulins in idiopathic hypothyroidism, which in this respect differed markedly from the postoperative group Of the 30 cases of idiopathic hypothyroidism the γ-globulin concentration was above the upper nor mal limit in 12. Markedly increased values of more than 1 8 g/100 ml were noted in 4 cases and in one it was as high as *2.23 g/100 ml.* No difference in this respect was found between the cases of *idiopathic hypothyroidism* with goitre and those without

Table II shows the number of positive T R C. tests (tanned red-cell agglutina tion) and C.F tests (complement fixa tion-capacity) in idiopathic hypothyroid-

Table III Erythrocyte sedimentation rate basal metabolic rate, serum cholesterol and serum protein pattern in case M J during treatment with thyroid

Year of exam.	ESR mm/1 hour	BMR %	Serum chol. mg/100 ml	Serum protein values g/100 ml Total	Alb.	α-glob.	α_2-glob.	β_1-glob.	β_2-glob.	γ-glob.
1951	140	—24	236	9.7	3.56		0.76	1.39		4.18
1954	90	—28	320	8.2	4.40	0.34	0.70	0.97		1.79
1956	75		281	7.6	3.97	0.42	0.67	0.48	0.36	1.70
1958	72	—	—	7.5	3.78	0.38	1.11	0.63	0.55	1.25

Table IV Serum protein patterns in family M J (See text)

Case	Diagnosis	Total	Alb.	α_1-glob.	α_2-glob.	β_1-glob.	β_2-glob.	γ-glob.
Mother	Hypothyroidism without goitre	9.7	3.56		0.76	1.39		4.18
Daughter	Hypothyroidism with goitre	8.0	4.67	0.43	0.65	0.52	0.56	1.39
Granddaughter 1	Euthyroid without goitre	7.6	5.27	0.33	0.49	0.47	0.24	0.80
Granddaughter 2	Euthyroid without goitre	7.8	4.85	0.34	0.59	0.52	0.29	1.11

ency was more marked in the individual cases. All patients who initially showed increased γ-globulin concentration and who were euthyroid at the review showed a decrease and the greater the original value the greater was the decrease. The 3 patients who initially had increased values and who showed no decrease during treatment, were hypothyroid. Two patients in whom the γ-globulin value before treatment was 1.93 and 1.84 g/100 ml, respectively had values of 1.38 and 1.55 g/100 ml, respectively after treatment, which were thus still clearly increased. These patients had however only been treated for 6 months, which is probably not long enough for a maximal effect of the treatment on the protein changes, even though the patients had become euthyroid clinically.

Erythrocyte sedimentation rate

Of the 44 patients examined 35 (79.5 %) had an ESR > 15 mm/1 hr and 9 (20.5 %) > 40 mm/1 hr. The mean of the entire material was 28 mm/1 hr (range 3—70 mm/1 hr). Among the various groups (table V) the mean value for the patients with pituitary hypothyroidism was lower than for those with thyrogenic hypothyroidism ($0.02 > p > 0.01$). No difference was found between the different forms of thyrogenic hypothyroidism.

The ESR has been shown normally to increase with age (34) but no such correlation was found in the present hypothyroidism series (fig 4 A). A fairly good correlation was found between the ESR and the protein-bound iodine (fig 4 B).

Table II Incidence of thyroid antibodies in idiopathic hypothyroidism compared with serum γ-globulin concentration.

γ-glob. conc.	No. of pat.	Positive serologic tests	
		T.R.C.	C.F
Increased	9	7	8
Not increased	15	11	5
Total	24	18	13

T.R.C. = Tanned-red-cell agglutination.
C.F = Complement fixation capacity
Th difference in C.F -test is statistically significant (χ = 6.46, $0.02 > p > 0.01$)

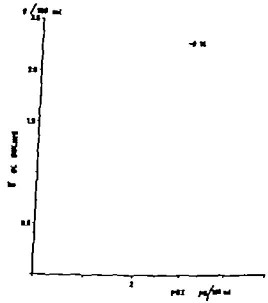


Fig 2. Correlation between serum protein-bound iodine and γ-globulin concentration in idiopathic hypothyroidism.
n = 26   r = — 0.69 $p > 0.2$.

period covered by this investigation. It is clear from the table that the γ-globulins decreased considerably Another 2 persons in this family 2 daughters of Mrs. M. J s daughter were examined but neither showed signs of thyroid disease and in both of them the serum proteins were normal (table IV) This family might be a variant of familial hypergammaglobulinaemia of the type described by Leonhardt (20)

Serum protein pattern in idiopathic hypothyroidism during treatment

Only patients with idiopathic hypothyroidism were studied after the institution of treatment. Of the originally 30 patients with idiopathic hypothyroidism 23 were re-examined The remaining 7 were not examined because the disease had only just been discovered in 2, 2 had stopped taking thyroid 1 had developed glandular tuberculosis, 1 had died and 1 had moved out of town. Of the 23 patients reviewed 8 had goitre.

Fig. 3 gives the various protein fractions before and after institution of treatment as well as the duration of treatment. The mean values before and during treatment in those cases who became euthyroid are inserted The albumin values showed an increase though statistically not significant. In 8 patients, 3 of whom were still hypothyroid, the increase noted was more than 10 % and in 5 4 euthyroid and 1 hypothyroid more than 20 %. A decrease of more than 10 % was noted in 2 of the patients, both euthyroid. The α_1 and α_2-globulin values showed no definite tendency The β_1-globulin values showed a statistically significant decrease in euthyroid cases ($p < 0.005$) In 7 cases, 1 hypothyroid and 6 euthyroid a decrease of more than 20 was noted while only 2 cases, one euthyroid and the other hypothyroid showed an increase of more than 20 %. The values of the β_2-globulins were not statistically changed When all cases were considered together the γ-globulins showed a moderate but not statistically significant decrease. The decrease was greater however in euthyroid cases ($p \to 0.05$) and this tend

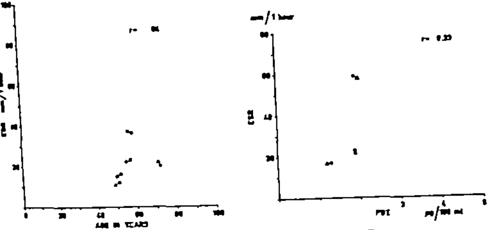


Fig. 4. A B

A. Correlation between ESR and age in years.
44 = + 0.25; $p > 0.2$.

B. Correlation between ESR and serum proteinbound iodine
= 40 t = − 2.39 $0.02 > p > 0.01$

○ idiopathic hypothyroidism
△ postoperative hypothyroidism
□ pituitary hypothyroidism

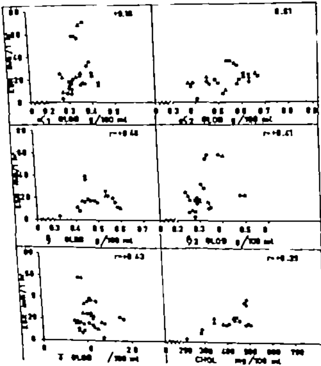


Fig. 5. Correlation between ESR and various serum protein fractions and between the ESR and serum cholesterol.

○ idiopathic hypothyroidism
△ postoperative hypothyroidism
□ pituitary hypothyroidism

α_1-glob.-ESR
= 44, − 1.18
$p > 0.2$

α_2-glob.-ESR
44 t = + 4.94;
$p < 0.005$

β_1-glob.-ESR
40, 3.37
$p < 0.005$

β_2-glob. ESR
40, 2.78
0.01 p 0.005

γ-glob. ESR
41, − 3.05
p 0.005

Cholesterol-ESR
42, 2.60
$0.02 > p > 0.01$

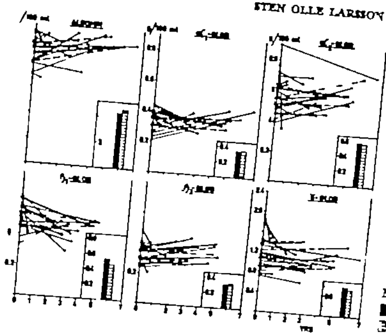


Fig 5. Serum protein fractions before and during treatment in idiopathic hypothyroidism. Inserted figures indicate mean values before and after treatment in those cases, who became euthyroid.

- ●——● idiopathic hypothyroidism with goitre — euthyroid
- ●– –● idiopathic hypothyroidism with goitre — hypothyroid
- ○——○ idiopathic hypothyroidism without goitre — euthyroid
- ○– –○ idiopathic hypothyroidism without goitre — hypothyroid

Mean values
■ before treatment
▤ after treatment

The correlation (fig 5) between the ESR and the α_2- β- and γ-globulins was very close. For the cholesterol and the ESR the correlation was fairly good ($0.02 > p > 0.01$)

The ESR was studied during treatment in the same group of patients as that in which protein electrophoresis had been performed. The ESR fell and the decrease was statistically significant ($p < 0.005$) (fig 6) If those cases be excluded in which the patients were not euthyroid at the review the difference will be still more marked Even in the euthyroid cases, however the mean value was still increased

Discussion

Using [131]I iodoalbumin in 6 cases of hypothyroidism Lewallen et al. showed increased outward transcapillary passage of albumin (18) Although they found no change in serum albumin the decreased values noted earlier and in 13 of the present 39 cases of thyrogenic hypothyroidism may be explained by such a mechanism. The tendency of the β_1-globulins to increase in the present material is certainly related to the fact shown by Laurell et al that it is the β_1-component of the β-globulins that is associated with the β-lipoproteins (17) which are increased in hypothyroidism (12)

Table 1 Sedimentation rate in various types of hypothyroidism

Aetiology of hypothyroidism	ESR > 15 mm	ESR > 40 mm	Mean mm/1 hr	Range mm 1 hr
Idiopathic with goitre	7/10	2 10	28	3—59
Idiopathic without goitre	18/20	5 20	31	12—70
Postoperative	7/9	2/9	30	9—68
Pituitary	3/5	0	18	8—29
Total	35/44	9/44	28	3—70

Observed range.

and with increased γ-globulin concentration, it was found that the C.F test but not the T.R.C. test was correlated with the γ-globulin concentration.

Lillington et al. recently published a paper on the ESR in different forms of thyrogenic hypothyroidism (21). Their series, which consisted of 60 cases, showed a mean ESR of 34.6 mm/1 hr (ranging from 5 to 74 mm/1 hr). As in the present investigation, no correlation was found between the ESR and age. Nor was the ESR found to be dependent on the aetiology of thyrogenic hypothyroidism, which was confirmed in the present investigation. Pituitary hypothyroidism, on the other hand seems to show lower values. In the Mayo Clinic material, which was divided into various groups according to the ESR values, the mean protein-bound iodine concentration was found to be lowest in the groups with the highest ESR, but owing to the smallness of the groups no statistical conclusions were drawn. In the present investigation fairly good correlation was found between the ESR and the protein-bound iodine concentration for the total material. This shows that the ESR is to a certain extent dependent on thyroid function, although it may be normal in some patients with a low protein-bound iodine concentration. As in the above-mentioned investigation, the present study showed that the ESR decreased during treatment, especially when the patients had become euthyroid. The reason why the mean ESR is still too high is probably that the ESR becomes normal much slower than the clinical manifestations of hypothyroidism (16).

Analysis of the data showed that the ESR was correlated not only with the r β- and γ-globulins but also to a certain extent with the serum cholesterol. The ESR is thus dependent not only on the serum proteins but perhaps also on the serum lipids.

The elevation of the ESR, which was sometimes considerable (20 / — > 40 mm/1 hr) underlines the importance of considering the possibility of hypothyroidism in patients with an obscure elevation of the ESR.

Summary

In an investigation of the serum proteins and ESR in 44 cases of hypothyroidism of varying aetiology the following observations were made

1 In thyrogenic hypothyroidism, both idiopathic and postoperative, the albumin tended to be low and the β_1-globulins tended to be increased the increase being less marked in the postoperative group.

2. The γ-globulins tended to be increased in idiopathic but not in postoperative hypothyroidism.

3. In 4 of 5 cases of pituitary hypothyroidism a reduction of the β_1-globulins was the only finding in an otherwise normal serum protein pattern.

4 In idiopathic hypothyroidism the serum proteins did not vary with the presence or absence of goitre.

5 No correlation was found between the γ-globulin and the protein-bound iodine in idiopathic hypothyroidism.

6 A significantly higher frequency of positive complement binding reaction was found in cases of idiopathic hypothyroidism with increased γ-globulin.

7 In 79.5 % of all cases the ESR was more than 15 mm/1 hr and in 20.5 % it was more than 40 mm, with a higher mean value for thyrogenic than for pituitary hypothyroidism but without any difference between the idiopathic and postoperative forms.

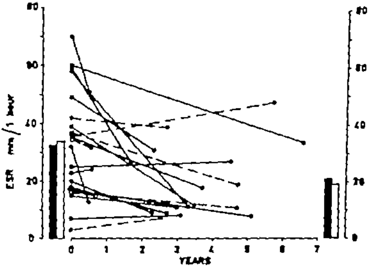


Fig. 6. ESR before and during treatment in idiopathic hypothyroidism. Mean values before and during treatment are inserted.
●——● idiopathic hypothyroidism with goitre — euthyroid
●– –● idiopathic hypothyroidism with goitre — hypothyroid
○——○ idiopathic hypothyroidism without goitre — euthyroid
○– –○ idiopathic hypothyroidism without goitre — hypothyroid
Mean values
■ still hypothyroid
□ euthyroid during treatment

The reason why the serum cholesterol is lower in pituitary than in thyrogenic hypothyroidism is, according to Skanse (29) that the thyroid function is not so severely impaired in pituitary as in thyrogenic hypothyroidism This might also explain the difference between pituitary and thyrogenic hypothyroidism regarding the albumin and β_1-globulin concentration

In about half of the present cases of idiopathic hypothyroidism the γ globulin level was elevated sometimes markedly In postoperative hypothyroidism on the other hand the γ-globulins were not increased.

The γ-globulin increase in hypothyroidism has been ascribed to the occurrence of thyroid antibodies (2) and already before the immunological era Luxton and Cooke claimed that they had found positive flocculation reactions in Hashimoto's disease and that these reactions were not due to hypothyroidism since they had also observed positive flocculation reactions in hyperthyroid and euthyroid patients (22) The poor correlation found in the present investigation between the serum protein-bound iodine and the γ-globulin and the difference in the γ-globulin concentration between idiopathic and postoperative hypothyroidism also argues for an increase in the γ-globulin being at least not essentially due to impaired thyroid function. However if in fact the increase in the γ-globulin is due entirely to the occurrence of antibodies against thyroid it is interesting that no difference was found between the patients with hypothyroidism with and without goitre. Since the development of goitre is an important part of the pathogenesis of Hashimoto's disease and since idiopathic hypothyroidism is supposed often to have the same immunological background one might perhaps, if the γ-globulin increase were due to thyroid antibodies, expect a higher concentration of γ-globulin in idiopathic hypothyroidism with goitre. It is also remarkable that the γ-globulin decreased during treatment in those patients who had become euthyroid for Skanse and Nilsson have shown that the antibody titre does not vary with the clinical picture or the duration of treatment (31) On comparison between the patients with idiopathic hypothyroidism with normal

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From the Medical Clinic of the University of Turku, Finland

The Effect of Blood Transfusion and Fluid Therapy on the Vital Capacity and Blood Circulation Time in Uremic Patients

By

Antero Kasanen and Risto Laheimaa

Anemia is a common secondary symptom in patients with renal disease. So far there is no specific therapy for it. Repeated blood transfusions have been regarded as the best method of treating it. Blood transfusion has had a beneficial influence on uremia in chronic nephritis. The patient's general condition improves and the symptoms of uremia abate (6, 13). The blood transfusion has had no detrimental effect whatsoever on renal function in spite of renal insufficiency (13).

There are however earlier reports to the effect that blood transfusion may prove a fatal measure (7). Four patients developed a pronounced pulmonary edema immediately after blood transfusion which in 3 of them ended fatally in 2 hours. The clinical picture was the same in all these cases. The patients had no earlier history of dyspnea and they had tolerated other fluid therapy well, but pulmonary edema developed rapidly during blood transfusion.

It is known, on the other hand that pulmonary changes occur in chronic renal diseases, the so-called uremic lung picture (12). It is often demonstrable roentgenologically as cloudy shadows confined to and most prominent in the inner zones of the lung fields, extending radially from the hilus, the peripheral part of the lungs being relatively free (5).

Material and methods

The authors gave 8 control patients 400 ml of blood. Eight uremic patients were given 400 ml of blood, 8 received only red blood cells which were obtained from the same quantity of blood and 8 patients received 800 ml of isotonic sodium bicarbonate (1.3 %). The infusion time was 2 hours. Vital capacity was measured 3 times in all the cases both before and after fluid therapy and the greatest value was accepted. The same person performed all the measurements. The blood circulation time, arm to tongue, was also measured both before and immediately after the test, using 3 ml of decholin. None of the uremic patients examined had shown pulmonary or cardiac changes roentgenologically before the transfusion. The control group was also selected to exclude hypertonia and cardiac insufficiency. As one patient of the uremic group died of pulmonary edema immediately after the transfusion, it was not considered advisable to enlarge the series.

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8 Correlations were found between the ESR on the one hand and the α_1 β- and γ-globulins and the serum cholesterol on the other

9 The ESR and the γ-globulins decreased during treatment, most markedly in those patients who became euthyroid.

10 The clinical manifestations of hypothyroidism diminished faster than the ESR and the γ-globulin concentration.

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the transfusion and profuse moist rales were heard in both lungs. Therapy consisted of hypertonic glucose and cedilanid intravenously; the patient seemed to respond at first, but had new attack of pulmonary edema at night and died of pronounced dyspnea.

Red cell transfusion and bicarbonate infusion

No change in vital capacity was noted when the patient was given red blood cells or bicarbonate solution (table II). Blood circulation time was also unchanged.

Discussion

It appeared from the experimental series that blood transfusion changes considerably the vital capacity of a uremic patient. In some cases the transfusion may cause pulmonary edema. One case ended fatally but Kasanen (7) has previously described 3 other cases with a fatal outcome. In contrast, vital capacity is not affected by red cell transfusion or ordinary fluid therapy.

It has been reported previously that the uremic lung is due primarily to an accumulation of fluid (1 14). Capillary permeability may have a role (3 4). A defect also in the fibrinolytic enzyme system of the lung tissue was established on post mortem in renal failure (9).

The authors regard local pulmonary changes, permeability disorders and inhibition of the fibrinolytic enzyme as the most important causes of accumulation of fluid in the alveoli. Hypervolemia would then be only the trigger mechanism.

It must be remembered, moreover that the uremic patient tolerates anemia, even severe anemia surprisingly well (10) and that stored blood contains plenty of potassium which is harmful for many renal patients.

The authors consider blood transfusion to be contraindicated in a uremic patient according to their findings. Cobalt is the best therapy and very effective even in individual cases (8). Red cell transfusion can, however be contemplated in some cases. It must be performed very slowly over the course of 4—5 hours and the patient must be kept in a sitting position throughout.

Summary

Blood, red blood cells and bicarbonate were given to patients with renal disease of varying severity. The control group was given whole blood as well. Each group consisted of 8 patients. Vital capacity and blood circulation time were measured before and after the infusion.

A reduction, average 0 36 occurs in the vital capacity of uremic patients after the whole blood transfusion irrespective of the severity of the disease. One of the patients died of pulmonary edema immediately after the transfusion. The red cell transfusion, bicarbonate infusion and the whole blood administered to the controls caused no changes.

The authors are of the opinion that the anemia of uremic patients should not be treated with blood transfusions but with cobalt. Red cell transfusion could be performed in desperate cases.

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Table I The effect of blood transfusion on the vital capacity of uremic patients and controls

Control group			Uremic group				
Vital capacity l			Serum creatinine mg %	Hb g %	Vital capacity l		
Before	After	Difference			Before	After	Difference
2 10	2 47	+ 0.37	14 0	6.0	4.24	3.67	− 0.57
3.74	3.71	− 0.03	10 0	7 4	3.02	2.53	− 0.49
3.64	3.50	− 0.14	4.0	6.6	2.24	2.04	− 0.20
3.22	3 18	− 0.04	3.8	8.7	2.30	2.20	− 0.10
2.90	2.90	0.00	3.6	8.5	3.03	3.19	− 1.84
3.10	3.20	+ 0.10	2.9	10 1	3.50	3.20	− 0.30
3.15	3.02	− 0.13	2.0	9 7	4.19	3.53	− 0.66
2.84	2.98	+ 0.14	1.5	7 0	2.27	2.06	− 0.21

$\bar{x} = -0.03 \pm 0.03$ $\bar{x} = -0.36 \pm 0.08$

This case has not been taken in the statistical analysis because the change was very great.

Table II The effect of red cell transfusion and intravenous infusion of bicarbonate on the vital capacity of uremic patients

Bicarbonat infusion VC			Red cell transfusion VC		
Before	After	Difference	Before	After	Difference
3 85	3 63	− 0.22	3.53	3.68	+ 0 15
3.07	2.84	− 0.23	2.46	1.99	− 0.47
3.29	3.50	+ 0.21	2.84	2.90	+ 0.06
2.34	2.48	+ 0.14	3.21	3.51	+ 0.30
1.93	1.82	− 0 11	2.54	2.32	− 0.22
3.92	3.53	− 0.39	2.29	2.11	− 0 18
3.96	3.72	− 0.24	3.84	3 92	+ 0.08
2.86	2 70	− 0.16	2.42	2.22	− 0.20

$\bar{x} = -0.13 \pm 0.07$ $\bar{x} = -0.06 \pm 0.19$

Results

Whole blood

No difference in vital capacity after the blood transfusion was established in the control group (table I) Nor were there any changes in the circulation time. The mean of the circulation time before blood transfusion was 20 sec and after transfusion 19 sec. It has previously been found that the blood circulation time does not change after the infusion of 1 500 ml of NaCl and that vital capacity diminishes by 5 % at the most (2)

All the uremic patients showed a distinct reduction in vital capacity It averaged 0 36 l. The decrease in vital capacity was not dependent on the degree of anemia or retention. The difference was significant $p < 0.01$ In one case the change was very great — 1 84 this has not been included in the statistical analysis. No changes were demonstrated in the blood circulation times.

Case 1 The patient was a boy of 16 who had had insidious general symptoms for 2 years. Chronic nephritis was diagnosed. Riva-Rocci was 180/125 when the blood transfusion was commenced. ECG revealed no signs of hypertrophy of the left ventricle and the lung roentgenogram was normal. Hb was 6 g and serum creatinine 14 mg %. Serum calcium was low 6 mg but other electrolytes were normal. The patient developed a violent asthmatic attack immediately after

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Endocrine Adenomatosis (Polyendocrine Disease)

Familial Occurrences

By

T. S. Danowski, Frank M. Mateer and Edward E. Longabaugh

By 1953 according to Underdahl et al. (75) multiple endocrine adenomas of the pancreatic islets, the parathyroids, the anterior pituitary and occasionally other endocrines had been reported in various combinations in 15 or more patients. Of the 8 new cases which they presented a peptic ulcer or gastric polyposis was noted in 4. Three of these also gave a history of peptic ulcers in other members of the family. In 1953 and 1954 Moldawer et al. (8, 49) reported a father and daughter with parathyroid adenomas and hyperparathyroidism. The daughter also had hypoglycemia while duodenal and stomal ulcers were present in the father his brother and his mother. Wermer (80) then described multiple endocrine adenomas and peptic ulcers in a father and four daughters. In most but not all instances summarized by Underdahl et al. and by Wermer the tumors appeared to be hypersecretory, i.e. hypoglycemia, hypercalcemia, hypophosphatemia, and acromegaly with or without diabetes mellitus were present.

Submitted for publication May 11, 1962.

In 1955 Zollinger and Ellison (82) described two females with tumors of the pancreatic islets and ulcers of the upper gastrointestinal tract. These patients differed from those in the above compilation of endocrine adenomatoses in that the ulcers were multiple, associated with large volumes of highly acid gastric secretions, and poorly responsive to antiulcer regimens. One of the two women resembled the endocrine adenomatosis group in that hypoglycemia was present. The other gave a family history of a duodenal and a fatal gastric ulcer in the mother and the father respectively and the death of a sister in spontaneous hypoglycemia. Since then approximately 130 examples of the Zollinger Ellison syndrome have appeared or been identified in the older literature (1, 3, 5-23, 25—27, 29, 31, 32, 34, 35, 37—40, 42—54, 56, 57, 59—66, 68, 71—84).

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Table I Findings in our familial example of adenomatosis

	Brother age 37 (Fisher & Flandreau (23))	Brother age 31 (Weber et al. (79))	Sister age 42 (Danowski et al.)
History			
Abdominal pain	+	+	
Vomiting	0	+ (3 mos)	
Diarrhea	Watery (1 yr)	+ (2 yrs)	
Melena	72 ×	0	
Weight loss	30 lbs	30 lbs	10 lbs
Renal colic	Several episodes at age 35		Calculus at age 27
Findings			
Gastric volume		*18 l/12 hrs*	
Gastric HCl		116.5 mEq/12 hrs	
G-I ulcer	Duod. (2) *Jejun. (1)*	Duod. with perfor	0
G-I hemorrhage		Massive 2 & 3 wks post-op.	
Serum K		3.5 mEq/l	
Endocrines			
Pituitary		Basophils & amphophils ↑	
Parathyroid		*Hyperplasia, S. Ca 11 4 mg %* S. phos 2.5 mg %	*Hyperplasia (2),* S. Ca 13.4 phos 2.0 mg %
Pancreas	*Cystic & nodular many alpha* cell tumors	*Alpha cell tumors, Bl. sugar 72*	
Adrenals		Hyperplasia	
Gonads		Atrophy	
Out-come	Pancreatectomy duodenectomy splenectomy subtotal gastrectomy died after cholecystectomy for acute cholecystitis, nephrocalcinosis	Repair of perfor ulcer with hemorrhage in 2 wks, total gastrectomy & excision alpha cell adenoma of pancreas with hemorrhage in 2 wks; resection body & tail of pancreas; died	Parathyroidectomy with apparent cure

In these various reports of endocrine adenomatoses eleven familial examples have been described *(23 45 47—49 56 65 72 79 80 82)*. We can now add a report of parathyroid hyperplasia and hyperparathyroidism in one of these eleven families. This occurred in a sister of the two brothers with Zollinger Ellison syndrome described separately by *Fisher* and Flandreau (23) and by Weber et al. (79)

Material

The clinical and laboratory information on the two brothers reported by Fisher and Flandreau (23) and by Weber et al. (79) is summarized in table I together with the data on the sister. It is to be noted that both brothers had gastrointestinal ulcers, diarrhea and islet cell tumors. Hyperparathyroidism *may also have been present in both males in* that the *younger one had hypercalcemia,* hypophosphatemia and enlarged parathyroid glands, *while the older one had* a history of renal colic and nephrocalcinosis at autopsy

The sister gave history of renal calculi and two hyperplastic parathyroid glands were removed. She had no gastrointestinal symptoms nor abnormal radiologic findings and there was no hypoglycemia. Also, glucose tolerance and insulin tolerance tests were normal in this patient.

The ten other familial examples of endocrine adenomas and a compilation of clinical findings in the familial and non-familial cases are shown in figs. 1 and 2 and table II.

Results

The examples of endocrine adenomatosis recognized to date can be placed in three largely chronologic groups. The first consists of the 29 patients assembled by Underdahl et al. (75) Moldawer et al. (8, 49) and by Wermer (80) prior to 1954 in whom the adenomas were frequently secretory and peptic ulcers were occasionally noted. The second comprises most of the examples of the Zollinger-Ellison syndrome in which intractable peptic ulceration in association with islet cell tumors was emphasized and occasionally other endocrine tumors, often deemed to be non-secretory were described. The third group is made up of the cases of islet cell tumors with diarrhea assembled by Verner and Morrison (76) Telling and Smiddy (73) and Morrison et al. (51) from their own experience and the literature.

Of the eleven familial examples of endocrine adenomatoses reviewed in the present communication two fall into group I and the remainder are in groups II and III.

The incidence in groups I, II and III of peptic ulcer diarrhea, renal calculi, hypoglycemia (blood sugar levels less than 60 mg/%) hypercalcemia (serum calcium level of 11 mg/% or higher) hypokalemia (serum potassium concentrations below 3.5 mEq/l) and the frequency of the various endocrine tumors are summarized in table II. It is obvious that there is considerable overlap of findings in these three groups with respect to most of the items in table II. Two exceptions can be cited i.e. the absence of diarrhea and steatorrhea in group I and the absence of hypoglycemia in group III.

Discussion

The occurrence of parathyroid hyperplasia and hyperparathyroidism in a sister of two brothers with islet cell tumors, peptic ulcers and diarrhea emphasizes that the cases described by Underdahl et al. (75) Wermer (80) Zollinger and Ellison (82) Moldawer (8, 48, 49) Verner and Morrison (76) Telling and Smiddy (73) Morrison et al. (51) and others are varying manifestations of a spectrum of endocrine disturbances which may be familial. To date the islet cell tumors, benign or malignant, single or multiple, and with or without peptic ulcers, hypoglycemia or diarrhea have been the most common, occurring in 75 per cent or more of the patients. The parathyroid adenomas have been the next most frequent (in 30 per cent) and judging from the serum calcium levels or other indices the majority of these do not result in identifiable hyperparathyroidism. The third most frequent adenoma in this collection of patients has been found in the anterior pituitary. Again, evidence of overactivity such as acromegaly was present in only a minority. Other endocrines have only rarely been involved. The few thyroid adenomas reported include one case each of thyroid cancer (65) and thyrotoxicosis (72). Also, there are

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G-I ulcer	Duod. (2) Jejun. (1)	Duod. with perfor.	0
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Serum K		3.5 mEq/l	
Endocrines			
Pituitary		Basophils & amphophils ↑	
Parathyroid		Hyperplasia, S. Ca 11.4 mg %, S. phos 2.5 mg %	Hyperplasia (?), S. Ca 13.4, phos 2.0 mg %
Pancreas	Cystic & nodular, many alpha cell tumors	Alpha cell tumors, Bl. sugar 7?	
Adrenals		Hyperplasia	
Gonads		Atrophy	
Out-come	Pancreatectomy, duodenectomy, splenectomy, subtotal gastrectomy; died after cholecystectomy for acute cholecystitis, nephrocalcinosis	Repair of perfor. ulcer with hemorrhage in 2 wks, total gastrectomy & excision alpha cell adenoma of pancreas with hemorrhage in 2 wks; resection body & tail of pancreas; died	Parathyroidectomy with apparent cure

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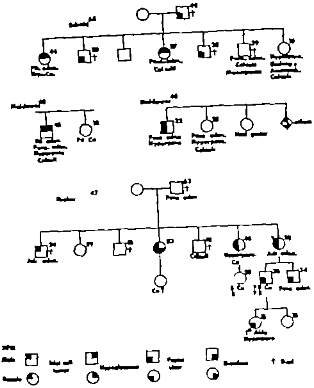


Fig. 2. These four families together with those in fig. 1 and the family described in this paper make total of 11 examples of familial endocrine adenomatosis or polyendocrine disease

Tumor tumors or adenomatous hyperplasia				Blood sugar (<60)	S. Ca (>11)	F (<3.5)	Renal calculi
Parathyroid	Thyroid	Adrenal	Islet cell				
23/29	2 29	4/29	15/29	12/29	11/29	1/29	5/29
30 72	3 72	6 72	37/72	8/72	10/72	1/72	6/72
		1/24	24/24		1/24	9/24	1/24

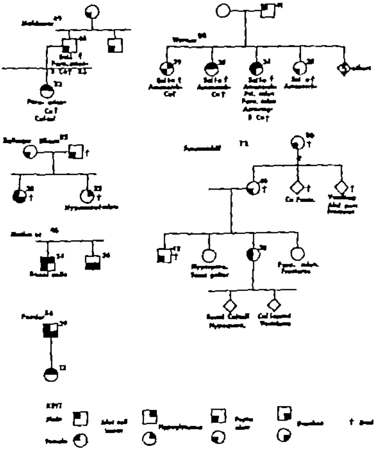


Fig 1 First six examples of familial endocrine adenomatosis or poly-endocrine disease with or without gastrointestinal disease (peptic ulcer and/or diarrhea)

Table II Denominator indicates total cases numerator identifies number of affected individuals

	Peptic ulcer	Diarrhea	Pituitary tumor	Acromegaly
Group I Underdahl et al. (75) Moldawer et al. (8, 49) Wermer (80)	10/29	0	11/29	11/29
Group II Zollinger & Ellison (82) Literature (cf 1 3 5 7 9 12, 15, 18 19 21 25—27 31 35, 37 38, 43, 45, 47 48, 52—54, 56, 59—61 63, 65, 71 74 77 78, 81 83, 84 in article)	60/72	16/72	8/72	1/72
Group III Telling & Smiddy (73) Morrison et al. (51)	12/24	24/24	1/24	

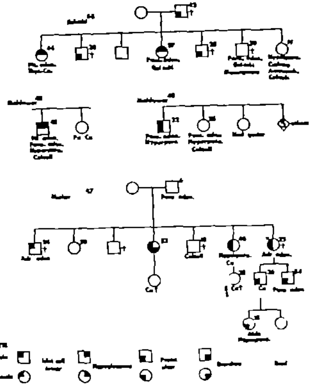


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Tumor tumors or adenomatous hyperplasia				Blood sugar (<60)	S. Ca (>11)	K (<3.5)	Renal calculi
Parathyroid	Thyroid	Adrenal	Islet cell				
23/29	2/29	4/29	15/29	12/29	11/29	1/29	5/29
18/72	3/72	6/72	57/72	8/72	10/72	1/72	6/72
		1/24	24/24		1/24	0/24	1/24

only a few examples of adrenocortical adenomas but none of these have been associated with definite evidence of hyperfunction. There is, however one patient described by Schmid et al. (65) in whom Cushing's syndrome of unknown origin (and therefore conceivably due to an adrenocortical or pituitary adenoma) was present. Moreover Miecher et al. (47) have described a kindred with endocrine adenomatosis and peptic ulcer in one of whom primary aldosteronism *perhaps arising from an adrenocortical* adenoma was found To date there have been no instances of pheochromocytoma or any type of ovarian or testicular tumor

Should such endocrine adenomatosis be called the Underdahl, the Moldawer the Wermer the Zollinger Ellison, or some other syndrome? Such eponyms *are often helpful as mnemonic devices.* Priority of detection or publication has little bearing on the question of selecting an eponym. One need only recall that Flajani (24) and then Parry (55) preceded Graves (33) in delineating thyrotoxicosis, that the Fröhlich (28) syndrome was described earlier by Babinski (2) that *Ponfick (58) Glinski (30) and Simmonds* (69) each antedated Sheehan (67) in defining various types of hypopituitarism, that Höring (36) and then Stör (70) anticipated Laurence and Moon (41) in calling attention to the concurrence of findings in a syndrome to which Biedl's (4) name was added much later etc. The *list is legion,* but enough has been cited to indicate that eponyms prematurely used can interfere with the adequate identification and communication. *Hence,* until the full spectrum of manifestations of endocrine adenomatosis or polyendocrine disease is defined these terms are to be preferred to the overlapping eponymic subtypes.

Summary

The reported *instances of multiple* endocrine tumors (endocrine adenomatosis or polyendocrine disease) suggest that a broad spectrum of manifestations may occur in which the Zollinger Ellison syndrome is but one example. Amongst the combinations recognized to date islet cell adenomas or cancers with or without *hypoglycemia, peptic ulceration, and* diarrhea have been first in frequency followed by functioning and non-functioning tumors of the parathyroids, anterior pituitary and occasionally of the thyroid and the adrenal cortices. An example of hyperparathyroidism in a female whose two brothers died with islet *cell tumors, peptic ulcers, and diarrhea* further emphasizes that the disorder can be familial. This has been noted in a total of eleven families in the approximately 130 cases described to date.

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The Routine Use of Quinidine in Myocardial Infarction

By

Steffen Hvidt, Birgit Blatt and Ragnhild Hvidt

On the consideration that an apparently harmless arrhythmia may occasionally represent the premonitory symptom of a rhythmic disturbance which may be fatal, Scherf (5) has recommended the use of quinidine in all cases of myocardial infarction.

However, according to the available literature clinical experience is sparse regarding this prophylactic therapy. Cutts and Rapoport (2) used dosages of varying degree in 111 patients with myocardial infarction who in addition received dicoumarol, but these investigators found no significant reduction either in the incidence of arrhythmia or in the rate of mortality. Boone and Pappas (1) found a fall in the rate of mortality viz. from 35 to 16 %, after administration of quinidine. These authors based their finding on 63 cases of infarction; however, some of the patients had been treated by anti-coagulating agents, and others had not.

The object of the present study is to elucidate the prophylactic effect of quinidine on mortality and on disturbances of heart rate, rhythm and conduction, within a period of three weeks following the onset of the acute myocardial infarction.

Material

The material is divided into two groups. The first group includes the quinidine treated patients, the second an untreated control group.

Group I. Patients admitted to the hospital within the first 24 hours following the occlusion who have been treated by 200 mg of oral quinidine t. i. d. combined with administration of anti-coagulants. This treatment was instituted within 24 hours following admission and continued for at least 21 days. Patients who died within the first 24 hours are not included, nor are patients who had received quinidine before admission or patients who have been treated by digitalis.

The diagnosis of myocardial infarction in this study was established on the basis of

a) Electrocardiographic evidence of recent myocardial infarction together with a medical history of typical chest pain, or

b) Definite findings at autopsy of myocardial infarction together with a typical history of pain.

Submitted for publication May 14, 1962.

Table I Composition of material

	Quinidine treated patients	Controls
	No. of infarctions	
	109	103
Male patients < 60 years	45	37
Male patients > 60 years	50	52
Female patients < 60 years	3	3
Female patients > 60 years	11	11
Patients presenting previous histories of infarction	31	23
Patients who at admission presented cardiac insufficiency	28	20
Patients presenting marked adiposity	22	16

The exact time of occurrence of the infarction has been determined exclusively on the basis of the anamnestic symptom of chest pain.

One hundred and nine infarctions diagnosed in 101 patients were found to conform to these criteria among patients admitted to the hospital within a period from June 1 1958, at which time the routine use of quinidine was introduced, to Dec. 15 1960 when the routine treatment using anti-coagulants was discontinued.

Group II The controls include patients with myocardial infarction treated by anti-coagulants but not by quinidine. These patients were selected on the same criteria as the ones in the former group, from male patients admitted in the period Jan. 1 1954 to May 1 1958, and from female patients admitted in the period May 1 1956, to May 1 1958. Thus the two groups were of about the same size. Patients above the age of 60 years did not appear in the group of quinidine treated cases and hence, this age group has been precluded also in the control group.

As regards sex and age distribution it will be noted from table I that the two groups are comparable. Conversely patients presenting previous histories of myocardial infarction cardial insufficiency and marked adiposity predominate in the quinidine group.

The control of patients has been equally rigid in the two periods, evaluated on the basis of number of electrocardiograms made per infarction within the 21 days following admission (9.3 in the quinidine group, 8.6 in the control group)

Indeed, the deviation as to time of admission in the two groups is rather adverse to comparison, but since the general rules of treatment have been uniform throughout, as already mentioned, we have found it justifiable to disregard time as a factor of decisive importance as regards comparison of the two series.

The general lines of treatment of myocardial infarction have remained unchanged throughout the periods of control and of quinidine therapy In particular the time and degree of mobilisation of patients has been the same, i. e. a night-commode has been used from the first day patients have been allowed to sit in bed-chairs as soon as shock had subsided and in ordinary chairs after three weeks of hospitalization after four weeks the patients have had permission to walk in the hospital passages.

Therapy by anti-coagulating agents (Heparin Marcoumar(®)) has been controlled by the laboratory of the hospital and hence one — never more than two — physicians have been responsible for dosages administered to the individual patients.

The prothrombin level was maintained, preferably between 10 and 30 % determined by the prothrombin-proconvertion method as suggested by Owren for a brief interval, however the thrombotest method of Owren was used.

Results

Disturbances of heart rate rhythm, and conduction

One or several episodes of disturbances of rate, rhythm and conduction were seen in 53 % of the cases of myocardial infarction in the quinidine group as compared with 42 % in the control group.

However these figures are not directly comparable since changes recorded within the first 24 hours following admission

Table II. Electrocardiographically recorded disturbances of rate, rhythm, and conduction within 21 days after admission in quinidine treated group and control group of patients with myocardial infarction

	Quinidine group (109 infarctions)		Control group (105 infarctions)	
	Total	Onset after 24 hours	Total	Onset after 24 hours
Sinus tachycardia	12	4	10	4
Sinus bradycardia	8	4	9	5
Supra-ventricular extra systoles	12	9	12	6
Atrial flutter-fibrillation	(4)	(1)	(5)	(3)
Total of supra-ventricular changes without atrial-flutter-fibrillation	32	17	31	15
Ventricular extra systoles	20	15	16	9
Ventricular tachycardia, flutter or fibrillation	0	0	0	0
Total of ventricular changes	20	15	16	9
Partial A—V block	9	6	2	0
Total A—V block	6	3	1	1
Right bundle branch block	6	3	5	1
Left bundle branch block	10	0	3	0
Total of disturbed conduction	31	12	11	2
Total no. of disturbances (excl. atrial-flutter-fibrillation)	83	44	58	26

occurred either before or after the onset of infarction and without or under the influence of varying degrees of quinidine action. Hence these initial 24 hours are not included in the final evaluation.

Disturbances of rhythm, rate, and conduction recorded after the initial 24 hours of admission include changes demonstrable even within the initial 24 hours but persisting beyond this time limit and also changes occurring at a later stage. Only the latter with a solitary reservation, are included in the study.

The choice of this time-limit is justified by the fact that although a maximum concentration of quinidine in the blood admittedly is not obtained until after 72 hours (3) it must be borne in mind also that quinidine is rapidly absorbed from the gastro-intestinal tract for which reason a certain effect may be anticipated within a matter of hours.

In the quinidine group as well as in the control group a number of patients have been precluded from the evaluation on account, exclusively, of digitalization indicated by atrial flutter or fibrillation starting during the stay in hospital and making comparison of this type of arrhythmia impossible.

Table II shows the distribution of disturbances of rhythm, rate and conduction, arranged on the basis of the above mentioned criteria.

The overall number of changes recorded after the initial 24 hours but within a three-week-period is higher in

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One hundred and nine infarctions diagnosed in 101 patients were found to conform to these criteria among patients admitted to the hospital within a period from June 1 1958, at which *time the routine use of quinidine was in*troduced, to Dec. 15 1960 when the routine treatment using anti-coagulants was discontinued.

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As regards sex and age distribution it will be noted from table I that the two groups are comparable. Conversely patients presenting previous histories of myocardial infarction, cardial insufficiency and marked adiposity predominate in the quinidine group.

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Indeed, the deviation as to time of admission in the two groups is rather adverse to comparison, but since the general rules of treatment have been uniform throughout, as already mentioned, we have found it justifiable to disregard time as a factor of decisive importance as regards comparison of the two series.

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Table II shows the distribution of disturbances of rhythm, rate, and conduction, arranged on the basis of the above mentioned criteria.

The overall number of changes recorded after the initial 24 hours but within a three-week period is higher in

the quinidine group than in the control series, 44 and 26 respectively

The incidence of sinus tachycardia (heart rate above 100/min.) of sinus bradycardia (rate below 50/min) and of supraventricular extra systoles (isolated as well as multiple) was of the same order in the two groups.

Ventricular extra systoles (isolated as well as multiple) were more frequent in *the quinidine treated group of patients* than in the controls. Since this particular type of arrhythmia via a ventricular tachycardia, may develop into fatal ventricular fibrillation (5) the effect of quinidine in such cases *calls for the* greatest attention In the material under discussion no cases of electrocardiografically proven ventricular fibrillation occurred however two of the patients in the quinidine group and one in the control group died presumably from arrhythmia.

The incidence of disturbed cardiac conduction is higher in the quinidine group than among the controls. This applies in particular to the A—V block and could probably be ascribed to therapy quinidine being known to reduce the rate of conduction in atria as well as in ventricles. Since an A—V block may be associated with the Morgagni-Stokes-Adams syndrome hazards of sudden death will be increased.

Rate of mortality

The number *of deaths occurring* be tween the 2nd and the 21st day after the onset of infarction in the group treated with quinidine was 9 out of a total of 109 infarctions. In the control group of 103 infarctions *the number* of deaths was also 9

The category of patients to whom the highest interest is attached in this connection is the one in which the subjects died from "assumed arrhythmia" — i. e. death occurring unexpectedly within a matter of minutes and autopsy failing to demonstrate any other immediate cause of death. It has been mentioned that two of the patients in the quinidine group and one in the control series belonged to this category

Side-effects of quinidine

The side-effects of quinidine are few when the drug is administered in the dosage of 200 mg quinidine t. i. d. *Among* 232 patients treated by quinidine the therapy had to be discontinued in five cases because of presumed drug fever and in one case because of exanthema. Whether or not these symptoms could actually be ascribed to quinidine remains open to discussion.

Conclusion

Bearing in mind that this study is concerned only with the interval between the 2nd and the 21st day *following* the onset of myocardial infarction, and that cases of atrial flutter and fibrillation are not included together with the fact that a certain deviation between the two *groups prevails as regards the* time factor and composition, the conclusions to be drawn are

1) Routine administration of quinidine fails to prevent occurrence of extra systoles,

2) nor does quinidine prevent disturbances of sinus rate in cases of myocardial infarction.

3) The rate of mortality remains the same whether or not quinidine is administered and finally

4) the incidence of disturbances in cardiac conduction *is apt to be higher* after administration of quinidine.

Hence, the routine use of quinidine in myocardial infarction in the dosage used in this paper is not recommended.

Summary

One hundred and one patients, presenting a total of 109 myocardial infarctions, admitted within 24 hours following the acute onset of the infarction and treated for three weeks by anti-coagulants and quinidine t. i. d. are compared with 89 patients presenting a total of 103 myocardial infarctions treated by anti-coagulating agents but not by quinidine. The rate of mortality as well as the incidence of disturbances in rate and rhythm were not reduced in the quinidine group in which the highest incidence of disturbed heart conduction was seen. In the dosage of 200 mg t. i. d. the use of quinidine cannot be recommended as a routine treatment in cases of myocardial infarction.

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From the Institute for Research in Thrombosis (Chief: P. A. Owren, M. D.)
University Hospital, Oslo, Norway

The Peripheral Blood Flow in Intermittent Claudication

III. The Significance of Oscillometry

By

Leif K. Hillestad

By definition oscillometry is a method for recording the fluctuations of limb volume during a single cardiac cycle. These fluctuations are caused by the phasic inequalities between the arrival and departure of blood. They are commonly referred to as the volume pulse or the pulsatory amplitude.

At present a great number of oscillometers and oscillographs are available (2, 7, 18). Some of them represent modifications of the original method such as exercise oscillometry (5, 14), digital plethysmography (8), photoelectric plethysmography (11) and rheography (13).

However, regardless of the design of these instruments and of their application on limbs at rest or after exercise, they all reflect the same phenomenon, namely the pulsatory amplitude. Consequently their value in estimating the peripheral blood flow depends entirely on the extent to which the pulsatory amplitude indicates the actual arterial flow.

Previous reports have offered evidence that this is not always the case. Increased peripheral resistance has been shown to cause arterial backflow and augmented pulsatory amplitudes by a simultaneous reduction of the net arterial inflow (16, 20). In normal digits Goetz (8, 9) as a rule noted a good correspondence between the pulse volume and the arterial flow whereas this was the exception rather than the rule in cases with organic arterial disease. He naturally ascribed this to the presence of collateral flow. Furthermore other authors (3, 17) demonstrated that the blood flow at rest could be normal in ischemic limbs with lowered oscillometric readings. Ultimately Holling and Verel (12) showed that elevating the forearm might treble the pulsatory amplitude while it reduced the flow.

These observations lead directly into the urgent problem of oscillometry. How far can the oscillometric readings be trusted as indices of the peripheral arterial blood flow?

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These observations lead directly into the urgent problem of oscillometry. How far can the oscillometric readings be trusted as indices of the peripheral arterial blood flow?

Submitted for publication May 15, 1962.

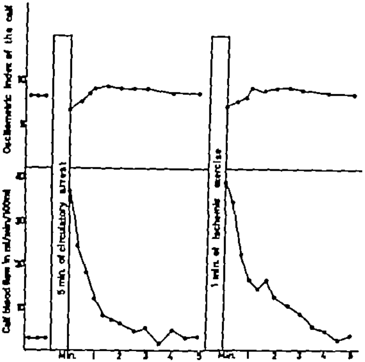


Fig 1 The relation between the oscillometric index and the blood flow of the calf in 5 normal subjects.

Table I Oscillometric readings and blood flow in a patient with congenital arterio-venous fistulas of the left leg Great fistula at the thigh and several small fistulas along the periphery of the limb

	Oscillometric index			Blood flow (ml/min/100 ml) of	
	At thigh	Below knee	At ankle	Calf	Foot
Left leg	15.0	8.5	4.5	128.0	66.8
Right leg	5.0	5.5	6.0	3.5	2.3

This problem is the subject of the present paper

The patients included in the study all suffered from only one disease namely intermittent claudication of the calf due to obliterative arteriosclerosis.

The study was done by comparing the oscillometric readings of the calf with the calf blood flow as obtained by plethysmography

Methods

Oscillometry

A Collens oscillometer (18) with single cuff and mechanical recording device was employed, because such simple models are ordinarily used in the clinics. The cuff was inflated to a pressure above the systolic and released stepwise to zero the deflections being noted at each step The greatest deflection was taken as the oscillometric index or reading

The oscillometer cuff was applied around the upper middle part of the calf just at the same level as the plethysmograph was placed. The examinations were done by the same investigator each time, and care was taken that the cuff was identically placed and adjusted at every examination.

Experimental room

By means of automatic control the room temperature was kept at 30° C to release the sympathetic tone of the vessels (10) The humidity was kept at around 50

Plethysmography

Venous occlusion plethysmography was used, the temperature of the water within the plethysmograph being 32 C. The blood flow of the calf was estimated at rest as well

as after circulatory arrest, ischemic and free exercise. The latter was obtained by pedalling an ergograph (10) at a fixed rate, which was signalled by a metronome. The arrest of blood flow lasted for 5 min., the ischemic exercise for 1 min. and the free exercise for 2 min. These procedures regularly created a calf hyperemia. The maximal flow value of this minus the resting flow is referred to in this paper as the peak flow or the capacity flow

Results

Clinically a vigorous pulsation of the arteries is most often regarded as consistent with an excellent blood flow through the same arteries. That this assumption of a parallelism between the magnitude of pulsation and flow is entirely fictitious can easily be demonstrated (table I) However the extreme vascular changes in arterio-venous fistulas do not justify a proper evaluation of the relationship between pulsatory amplitude and flow

It is more relevant to study the oscillometric readings in normal limbs during experimental alteration of their blood flow (fig. 1) It is thereby revealed that, contrary to the general belief, the index becomes reduced at high flows. The immediate impression is that an inverse relationship exists between index and flow However a closer analysis shows that the changes of index are negligible as compared with those of flow Immediately after release of the arrest, for instance there is an 1,800 % increase of the flow against a 25 % decrease of index.

Similar experiments on ischemic limbs are tabulated to allow a more detailed analysis (table II) The recording of the hyperemic curves were commenced within five seconds after cessation of the various procedures, and the curves are all typical for restricted arterial blood

Table II Blood flow and oscillometric indices of the calf during the hyperemia following circulatory arrest, ischemic exercise and free exercise. Average data from 11 limbs with intermittent claudication of the calf The left column indicates the times of hyperemia at which the flow curves are collected. Blood flow in ml/min/100 ml tissue

Time of recordings during hyperemia	Circulatory arrest 5 min.		Ischemic exercise 1 min.		Free exercise 2 min.	
	Flow	Index	Flow	Index	Flow	Index
5	13.1	0.5	13.7	0.6	13.0	0.8
20"	13.9	0.8	14.1	1.0	13.2	1.3
40"	12.0	1.3	14.5	1.3	12.0	1.6
1	10.9	1.7	13.0	1.5	10.9	2.1
20"	8.7	2.0	12.3	1.5	9.6	1.9
40"	4.7	2.2	11.1	1.7	8.7	1.9
2'	4.5	2.1	9.8	2.0	8.3	1.9
30"	4.3	2.1	8.8	2.0	7.7	2.2
3'	4.0	2.0	8.0	1.9	7.3	2.2
30"	3.8	1.9	8.0	2.0	6.5	2.2
4	3.3	1.8	7.4	2.0	6.4	2.3
30"	3.0	1.9	7.0	2.0	6.3	2.2
5	3.0	1.7	6.5	2.1	6.0	2.2
6'	2.7	1.8	5.9	2.1	5.5	2.2
7'	3.0	1.7	5.5	1.9	5.4	2.1
8'	3.0	1.7	5.2	1.9	5.1	2.1

flow They are protracted with subnormal and delayed peak flows. To reveal any difference in the behavior of the oscillometric readings between ischemic and free exercise, the duration of exercise was arranged so that the subsequent hyperemias became as similar as possible.

Although a certain inverse relationship exists between pulsatory amplitude and flow the changes of these two variables are not parallel in magnitude. A survey of the changes of index and flow at different stages of the hyperemia is given in table III It is obvious that it is hazardous to use the oscillometric readings as representative of blood flow under these

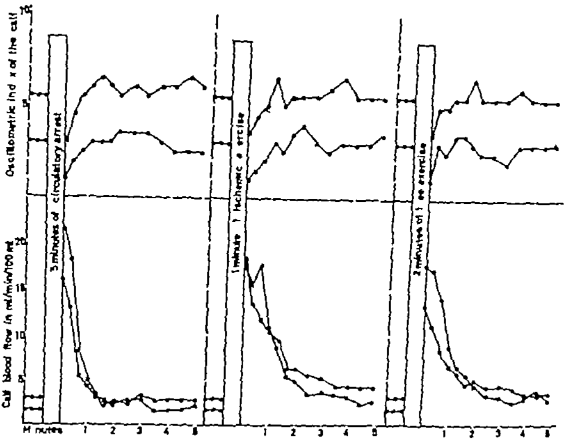


Fig 2 Oscillometric readings and hyperemic blood flows of the calf in two limbs with intermittent claudication of the calf.

○ = incomplete stenosis of the iliac artery ● = incomplete stenosis of the femoral artery

Table III Blood flow and oscillometric indices of the calf at different stages of hyperemia. The calculations are prepared from table II

Procedures used to produce the hyperemic blood flow	Changes of blood flow and index in % during hyperemia					
	Initial recording		During the first min.		During the 2nd to the 5th min.	
	Flow	Index	Flow	Index	Flow	Index
5 min. of circulatory arrest	+ 337	−73	−22	+ 240	−78	0
1 min. of ischemic exercise	+ 357	−68	− 8	+ 150	−64	+ 27
1 min. of free exercise	+ 300	−60	−16	+ 163	−63	0
Mean	+ 331	−67	−15	+ 184	−68	+ 9

circumstances. Moreover it should be noted that there *is no difference in this* respect between the ischemic and the free exercise.

The discrepancy between the two variables *is further illustrated in fig 2* The hyperemic curves are nearly identical, but the readings very different. The op-

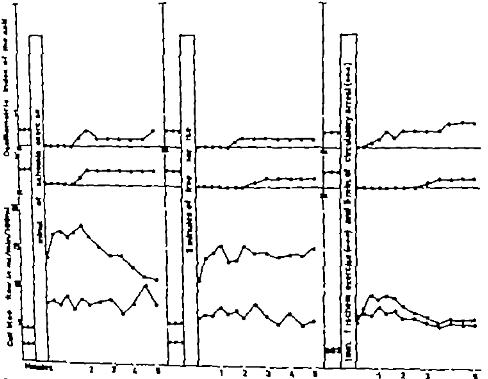


Fig. 3. Oscillometric readings and hyperemic blood flows of the calf in two limbs with intermittent claudication of the calf.

Complete stenosis of the femoral artery in both cases.

posite is found in fig. 3 as regards the post-exercise curves. Noteworthy is that the oscillometric deflections are absent for about the same time in spite of different magnitudes of flow. In contrast the hyperemias after arrest of the flow are similar but the recovery times for the pulsations widely different.

After this it is no surprise that the oscillometric readings are unable to indicate the calf blood flow at rest (fig. 4). In all probability the presence of weak pulsating collateral flow contributes to this result.

It is justified now to conclude that the pulsatory amplitude is not representative for the peripheral arterial flow and that oscillometry should not be employed for this purpose.

But it is not justified to abandon the use of oscillometry in the examination of ischemic limbs, for the following reasons. The student of vascular disease does not want information on the blood flow at rest, but rather on the capacity of the arterial circulation. This requirement is met by the peak flow or capacity flow which is obtained by plethysmography and which mirrors the degree of arterial insufficiency. The latter is roughly proportionate to the extent of arterial stenosis, which again is reflected by pulse palpation and oscillometry.

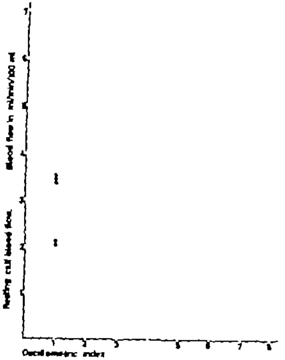


Fig 4 The relation between oscillometric readings and resting blood flow of the calf in a number of limbs with intermittent claudication.

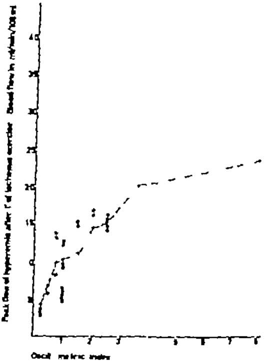


Fig 5 The relation between oscillometric readings of the calf and the calf blood-flow capacity (peak flow) in a number of limbs with intermittent claudication.

Table IV Oscillometric readings and calf blood flow in 10 limbs with femoral artery stenosis after successful reconstructive surgery

	Oscillometric index			Calf blood flow (ml/min/100ml)	
	At thigh	Below knee	At ankle	At rest	Peak flow
Before operation	2.0	1 0	1 0	3.5	10.5
After operation	4 0	6.5	4.0	5.2	23.0

The oscillometry may therefore provide data which suggest the capacity flow (fig 5) However the greater the proportion of collateral flow the less can these data be trusted. As seen from the figure the readings can be near zero and still allow a peak flow of about 10 ml/min/100 ml which is sufficient for an adequate nutrition of the tissues as well as for a walking distance of say 100—150 meters.

Major alterations of the circulation may be well revealed by means of oscillometry such as those following modern vascular surgery (table IV) Compared with flow studies, however oscillometric data as a rule give too favorable pictures of the improvement. This is apparently due to the presence of collateral flow before the operation a fact which therefore should be considered in this connection.

The evaluation of oscillometry would not be complete without an investigation of the spontaneous variability of the results, a question of fundamental importance for its use in controlling treatment. Such an investigation was there-

Table V. The spontaneous variation of oscillometric readings at the thigh in the course of 4 subsequent months. From 10 limbs with intermittent claudication

Months 1	2	3	4	G_o	S.d.	V.c.
4.50	3.50	3.00	2.00	3.50	1.47	42.00
2.00	1.25	0.75	0.50	1.10	0.67	60.00
1.50	1.25	1.00	1.00	1.20	0.67	55.83
8.00	6.00	6.00	7.50	6.90	1.03	15.00
7.00	7.00	7.00	6.50	6.90	0.24	3.50
1.50	2.50	1.00	1.00	1.40	0.71	50.70
2.50	2.50	1.50	2.25	2.20	0.47	21.30
0.75	1.00	1.00	2.00	1.20	0.56	46.67
7.00	5.50	5.50	4.50	5.60	1.03	18.40
1.50	1.00	0.75	1.00	1.06	0.31	28.90
Mean var. (indiv.) 3.7	3.1	3.1	2.8	3.1	0.74	34.1 %
Monthly var.(group) 3.7	3.1	3.1	2.8	3.1	0.39	12.6 %

Table VI. The spontaneous variation of oscillometric readings at the calf just below the knee. From 10 limbs with intermittent claudication

Months 1	2	3	4	G_o	S.d.	V.c.
3.50	3.00	5.00	3.25	3.67	0.89	24.20
0.75	0.50	0.50	0.25	0.50	0.20	40.00
2.25	2.00	1.00	1.00	1.56	0.66	43.60
1.00	0.50	0.50	0.75	0.69	0.24	34.29
0.50	0.50	0.50	0.75	0.56	0.14	25.00
1.50	2.25	0.75	1.00	1.38	0.41	29.28
2.00	2.00	1.50	2.00	1.88	0.24	12.63
1.50	2.00	2.00	3.00	2.62	0.84	32.31
6.00	6.50	6.50	6.50	6.38	0.24	3.83
0.25	0.75	0.50	0.75	0.50	0.24	48.00
Mean var. (indiv.) 1.9	2.0	1.8	1.9	1.9	0.4	29.3 %
Monthly var.(group) 1.9	2.0	1.9	1.9	1.9	0.05	2.63%

fore made on limbs whose blood flow remained stationary during the observation period (tables V, VI and VII). Briefly it must be stated that the mean individual variation of such readings (34.1 %, 29.3 ... 35.7 %) is so great that oscillometry has a restricted value for follow-up examinations of the single patient. For groups of patients the method can be employed within the limits indicated by the above findings (12.6 %, 2.6 %, 11.3 %).

Table VII The spontaneous variation of oscillometric readings at the ankle From 10 limbs with intermittent claudication

Months 1	2	3	4	$\bar{O}_a$	S.d.	V.c.
2.00	4.00	4 00	2.50	3.10	1 02	33.00
0.25	0.25	0.25	0.25	0.25	0.00	0.00
1.25	0.75	0.50	0.50	0.75	0.35	46.60
1.00	0.50	0.50	0.50	0.69	0.26	37 78
0.25	0.50	0.50	0.50	0.44	0.14	31.81
1 00	2.75	1.00	1.50	1.56	0.77	49.34
2.00	2.00	1 75	2.25	2.00	0.20	10 00
1.00	2.00	2.00	3.00	2.00	0.82	41.00
4 00	3.50	4.50	3.50	4.40	0.71	16.14
0.25	0.25	1.25	2.50	1 06	1.07	101.00
Mean var (indiv) 1.3	1.6	1.5	1 7	1.5	0.5	35.7 %
Monthly var (group) 1.3	1 6	1.5	1 7	1.5	0.17	11.33%

Comments

The problems concerning pulsatory amplitude *versus* blood flow are common to several vascular beds in the body In most of them the pulsatory amplitude is much easier to assess than is the flow and it is tempting to take the former as an index of the latter Unfortunately this is not correct

Confirming previous reports (3 17) this paper reveals that the oscillometer does not reflect the blood flow at rest in ischemic limbs. It also shows that the oscillometric readings are unable to indicate the hyperemic blood flow following circulatory arrest, ischemic and free exercise.

The experiments on post-exercise hyperemias constitute a corrective to the interpretation of data obtained by exercise oscillometry (5 6 14) Exercise oscillometry has been based upon the observation that the oscillometric deflections may disappear or become diminished for a while after exercise in ischemic limbs (1) The oscillograms are recorded before and after a standard amount of exercise, which consists of running on a staircase or riding a cycle ergometer The time elapsed after exercise until reappearance and normalization of the oscillometric deflections is taken as a measure of the peripheral blood flow The kind of exercise employed affects the cardiac output the blood pressure and vascular beds outside the ischemic limb under examination. It is therefore not the peripheral circulation proper that is estimated

The method used in this study is, however strictly regional, the muscles exercised being only those whose blood flow is to be estimated. There occur no appreciable changes of the systemic blood pressure or the cardiac output

Although these methodological differences are present it is probable that the demonstrated discrepancies between oscillometric readings and flow also must influence the significance of exercise oscillometry It becomes very difficult

to translate data obtained by this method into terms of blood flow

By means of exercise oscillography Ejrup (5) found that exercise improved the pulsations in normal limbs, whereas it reduced the pulsations in limbs with obliterative arterial disease. The latter pathologic response was by him termed the "inverse reaction" However occasionally he met this inverse reaction in normal limbs too, as a rule within the first 45 seconds after exercise. He therefore considered as truly pathologic only those inverse reactions that lasted longer than 60 seconds after the cessation of exercise.

McDonald and Semple (15) used the same method and demonstrated that the inverse reaction could be produced in normal limbs, provided the exercise was heavy enough. They suggested the pathologic inverse reaction to be merely an accentuation of a normal reaction.

Edwards et al. (4) noted an increase of the pulsations of the calf after exercise both in normal and arteriosclerotic limbs. The readings were made 1 to 2 min. after exercise, and this explains their results. As demonstrated in this paper (table II) the decreased pulsations of the calf correspond to the early phase of the hyperemia, whereas the pulsations are normal or above normal 1 to 2 min after cessation of exercise.

Wessor et al. (19) found the pulsations of the calf after exercise relatively unchanged in normal as well as in arteriosclerotic limbs. Their paper reveals that the post-exercise flows in normal calves were much greater at 1 min than at 15 sec. This is not the case when conventional venous occlusion plethysmography is employed on normal limbs, the highest flows being the ones obtained at 5 or 15 sec. after exercise. It is therefore possible that technical differences may be responsible for the discrepancy between their results and the findings of the present report.

The view presented by McDonald and Semple (15) is supported by this study There seems not to be any essential difference between the behavior of pulsations after exercise in normal limbs and in limbs with obliterative arterial disease. In both instances there occurred an initial decrease of the pulsations. It is therefore hardly correct to use the term "inverse reaction" as indicating a distinct pathologic phenomenon.

For the understanding of the problems concerning pulsatory amplitude versus flow it is, as mentioned by Rodbard and Jannotta (16) useful to consider Bernoulli's equation (18) According to this the flow energy is constant at a given perfusion pressure. The flow energy can be divided into a lateral pressure energy and a kinetic energy When one of them increases, the other must decline. Naturally Bernoulli's law is modified by various factors in the living body such as friction, tone and tension of the vessel walls and others. But nevertheless it is applicable and explains fairly good the results of this report on post-contraction hyperemia.

Rodbard and Jannotta (16) noted increased oscillometric deflections in the upper arm when a tourniquet was applied distal to the cuff. They could, however not find any changes of the deflections when the tourniquet was released. It is probable that the result would have been otherwise if they had done oscillometry at the forearm after release of the tourniquet at the upper arm.

It is demonstrated in this report that oscillometry is of no aid in estimating

the blood flow at rest in ischemic limbs (fig 4) Noteworthy is also that the figure reveals that the blood flow at rest in ischemic limbs is often above normal and that this is the case especially in the limbs with the most extensive obliterative disease. It is, however impossible from the figure to tell whether the increased resting calf flow is due to an increase of skin flow or muscle flow

In spite of the inability of oscillometry in assessing the blood flow proper the method should not be excluded from clinical use It may help in picking up pulsations where arteries are deeply situated or covered by edema assist in detecting arterio-venous fistulas and disclose differences of deflection at symmetrical levels of the limbs It also furnishes data in arbitrary units, which better than the pulse palpation suggest the capacity of the arterial circulation But when it is used on ischemic limbs the presence of collateral flow must always be considered.

Finally the great spontaneous variability of oscillometric readings under constant blood flow conditions is to be remembered This variation is readily understandable from consideration of the many factors determining the oscillometric deflections (10) But even with due consideration of these factors the variability of the readings at monthly intervals is great. The method therefore seems limited to detecting only major alterations of the circulation for follow up purposes.

In conclusion it must be stated that the pulsatory amplitude is not to be trusted as a representative for the peripheral blood flow In the examination of vascular diseases oscillometry should be regarded merely as a supplement to pulse palpation.

Summary

The oscillometric readings and the blood flow of the calf have been studied in limbs with intermittent claudication of the calf

The oscillometric readings gave no indication of the calf blood flow at rest. Nor did they reliably reflect the hyperemic blood flow following arrest of the circulation or ischemic and free exercise of the calf muscles.

There was no essential difference between normal and ischemic limbs in the behavior of the pulsations after exercise.

In ischemic limbs the oscillometric readings may as may the pulse palpation, give an idea of the capacity of the arterial circulation. But this idea becomes less correct the greater is the proportion of collateral flow present.

The monthly variation of such readings during unaltered flow was found to be of an order that renders oscillometry of restricted value in follow up studies of vascular disorders.

It is concluded that the pulsatory amplitude cannot be used as representative for the peripheral blood flow

In the study of ischemic limbs oscillometry should be regarded merely as a supplement to pulse palpation.

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Myocardial Infarction in the Younger Age Groups

III. Follow-up Observations with Special Reference to Exercise Tolerance Tests

By

Bror Söderholm, Olav Thulesius, Folke Heyman, Raoul Malmcrona and Per Björntorp

This is the fourth of a series (1, 8, 9) of reports concerned with the evaluation of data gained from a clinical review of case records and follow-up observations in a group of patients who have experienced myocardial infarction. The patients under consideration are all from younger age groups thus belonging to the working population. In these patients it was of special interest to get information as to the disabling effect of a myocardial infarction. Therefore, in order to provide some objective information about physical working capacity and the incidence of coronary insufficiency exercise tolerance tests have been performed. This report will consider in some detail the method and results of exercise tolerance tests performed at re-examination. Comparison will be made with other clinical data and the results of rehabilitation.

Material and methods

The original material comprises 318 cases of myocardial infarction up to the age of 55. For details of the original material and follow-up study see Malmcrona et al. (8, 9). The present study is based upon follow-up observations of 155 of these patients, 94 of whom performed an exercise tolerance test. The reason for excluding 61 patients from the exercise test was history of several infarctions or recent attacks of angina pectoris in 15 cases and in 41 instances markedly pathological signs in the electrocardiogram at rest, the pattern often being changed in comparison with previous tracings. Five cases refused the exercise test.

Exercise test

Exercise tests were performed with a bicycle ergometer designed by Holmgren and Mattson (6). In male patients the tests were started with a load of 300 kpm/min at a pedalling rate of 60 revolutions per minute. The heart rate and respiratory frequency were determined every two minutes. The work load was increased by 300 kpm/min when the patient had reached a circulatory steady state after 4—6 minutes. The heart rate was measured every two minutes. "Steady state" was considered to be present when the heart rate was constant or the increase in rate did not exceed 5 beats between two measurements. The exercise was discontinued when 1) the heart rate exceeded 160 beats/min, 2) any electrocardiographic abnormality or

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Table I Reasons for discontinuance of exercise test in relation to electrocardiographic response

ECG response on exercise	Reason for stopping test				
	Precordial pain	ECG changes	Heart rate 160/min	Fatigue	Total
Normal	4	2	6	40	52
Non-specific	2	–	1	6	9
Pathological	10	20	1	2	33
Total	16	22	8	48	94

Table II Electrocardiographic response to exercise in relation to electrocardiographic signs during hospital stay (acute stage of myocardial infarction) 3 cases excluded

ECG during hospital stay	ECG response to exercise			
	Normal	Non-specific	Pathological	Total
Q ± ST elevation	37	7	26	70
ST and T alterations	13	2	6	21
Total	50	9	32	91

Table III Electrocardiographic response to exercise in relation to resting electrocardiogram at re-examination

ECG at re-examination	ECG response to exercise			
	Normal	Non-specific	Pathological	Total
Normal	17	2	2	21
ST—T alteration	8	1	4	13
Abnormal Q	14	–	7	21
Q + ST—T alteration	13	6	20	39
Total	52	9	33	94

curred 3) the patient complained of chest pain or any other discomfort In female patients exactly the same procedure was performed except that the work loads were smaller beginning with 200 kpm/min at each period. In this way exercise at maximal loads of 600—900 kpm/min was accomplished in some cases, but in others the test had to be discontinued at smaller work loads.

During exercise precordial *H* leads (the reference electrode being attached to the front head) were obtained from the positions C_4, C_5, C_6 and C_7. Before the exercise was started a tracing was recorded with the patient recumbent and in the sitting position on the bicycle. Immediately after the exercise was stopped, and at 4 and 10 min after cessation of work, complete tracings were obtained including 3 standard extremity leads I II and III 3 unipolar extremity leads aVR, aVL, aVF and 5 chest leads. In the recumbent position R leads were always recorded.

The electrocardiographic response to exercise was considered *pathological* if there were

a) depression of the whole RS-T segment as a rule by more than 0.5 mm ("plateau depression") often with a preterminal downward sloping

b) accentuation of a previously pathological ST T pattern

c) transient arrhythmias (auricular fibrillation runs of premature ventricular beats), intermittent bundle branch block during work.

Electrocardiographic alterations classified as *non-specific* were

a) RS-T segment depression of minor degree (below 0.5 mm) without the characteristic contour and usually confined to the ST-junction

b) occasional ventricular premature beats at minor work loads or after work.

Pathological alterations during exercise usually persisted after the work test was discontinued and were often accentuated 4 min after work.

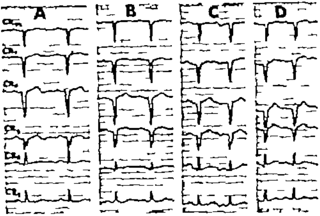


Fig. 1. Uncharacteristic electrocardiographic response in chest lead. A before exercise B sitting on the bicycle before exercise, C during exercise, D after exercise. For further details see text.

Results

Of the 94 cases who performed an exercise test, 21 had a resting electrocardiogram which was within normal limits. The remaining 73 cases had abnormal tracings. The electrocardiographic response to exercise was normal in 17 and 35 cases of the two groups respectively. A normal electrocardiographic response on exercise was thus present in a total of 52 cases, corresponding to an incidence of 55 per cent. Non-specific changes were noted in 19 cases (10) and a pathological response occurred in 33 instances (35 %).

In table I the various reasons for discontinuing the exercise test are listed in relation to the electrocardiographic response. Precordial pain has been a more common reason for stopping the exercise in the group with a pathological response than in cases with normal electrocardiographic findings. It should moreover be noted that the most common reason for interrupting the exercise tolerance test was fatigue.

In table II the distribution of pathologic and normal electrocardiographic responses to exercise tests is recorded in relation to electrocardiographic signs seen in hospital during the acute stage. One case with a normal electrocardiogram during the course of illness and at re-examination as well as 2 cases with broad and deep Q waves prior to infarction and at control examination have been omitted from this table. There was no significant correlation between the incidence of a pathologic response on exercise and major (Q ± ST elevation) or minor (ST and T alteration) electrocardiographic signs during the acute stage of the illness.

Table III shows the relationship between the electrocardiographic pattern at re-examination and the response to exercise. A pathological response was found more often in those cases with a combination of Q and ST T-alterations at rest than in those with an isolated Q wave.

Uncharacteristic electrocardiographic responses

In 18 cases who presented electrocardiographic signs of anterior myocardial infarction (with initial QRS-vectors displaying abnormal Q deflection in right-sided and transitional zone complexes together with deep negative T waves in the corresponding leads) negative T-waves became positive during the exercise test. In 11 of these cases this

Table II Electrocardiographic response to exercise in relation to anamnestic angina pectoris

Symptoms	ECG response to exercise			
	Normal	Non-specific	Patho-logical	Total
No chest pain	21	5	13	39
Uncharacteristic chest pain	7	2	3	12
Angina of effort	24	2	17	43
Total	52	9	33	94

Table I Electrocardiographic response to exercise in relation to capacity for work in 92 patients able to work prior to infarction

Capacity for work	ECG response to exercise			
	Normal	Non-specific	Patho-logical	Total
Ordinary	41	7	8	76
Limited	9	0	3	12
Unable to work	1	1		4
Total	51	8	33	9[illegible]

change appeared already in the sitting position on the bicycle ergometer before the work test was started during the actual exercise period a further normalization occurred A typical example is shown in fig 1 Usually the development of positive T waves was accompanied by a moderate ST-elevation in the same leads. This electrocardiographic pattern was not considered abnormal except in 3 cases where there was a concomitant depression of the ST segment in left-sided precordial leads.

Exercise test — angina pectoris

The electrocardiographic response to exercise in relation to the incidence of angina pectoris, as disclosed by follow up inquiries can be seen in table IV It must be mentioned that 35 cases had to be rejected in the category of patients with angina pectoris (24 because of markedly pathological changes in the electrocardiogram already at rest and 11 on account of recent attacks of severe anginal pain) In those cases where an exercise test could be performed angina pectoris was with equal frequency found in the group with a normal response on exercise and in the group with a pathological electrocardiographic response.

In about 50 % of the cases with abnormal exercise tests, chest pain was reported during the test. On the other hand precordial pain occurred in less than 10 % of the cases with a normal test

Exercise test — capacity for work

Table V shows the electrocardiographic response to exercise in relation to capacity for work and employment The number of cases rejected from the exercise tests increases with decreasing capacity for work. Among the patients with ordinary working capacity 76 % were considered able to perform the test and of those who were unable to work only 4 of 18 could be accepted for the exercise test. A comparison between the cases with an ordinary capacity for work and those with a more or less limited capacity for work who were tested yielded no significant difference as to the electrocardiographic response on exercise.

Physical working capacity

Table VI supplies information as to mean heart rate values at different work loads. For purposes of comparison corresponding values for a control group of 115 healthy male subjects are added

Table VI. Heart rates at different work loads in patients and controls grouped according to the maximal work load performed in "steady state"

Work load kpm/min		Heart rates at submaximal work loads					
		"300"	"600"		"900"		"1,200"
		Infarcts	Controls	Infarcts	Controls	Infarcts	Controls
300	M	112	112	100	104	100	97
		3	3	3	2	3	2
		26	15	29	57	12	43
600	M	–	144	127	130	120	119
		–	4	3	2	4	2
		–	15	29	57	12	43
900	M	–	–	–	155	141	145
		–	–	–	2	4	2
		–	–	–	57	12	43
1,200	M	–	–	–	–	–	169
		–	–	–	–	–	1
		–	–	–	–	–	43

M = mean values = standard error of the mean n = no. of cases.

derived from Grimby and Söderholm (5). Only men will be considered, because the female group is too small for further analysis. No attempt has been made to study the maximal working capacity.

Those patients who only reached 300 kpm/min as the highest work load had significantly higher heart rate at this load than the cases who reached 600 kpm/min or more ($p < 0.01$). There was no significant difference between the heart rates at the different work loads of those who reached 600 kpm/min or more. The heart rates of these patients were significantly lower than those of the controls ($p < 0.01$). There were no differences in heart rates in patients with or without angina pectoris.

Discussion

The re-examinations in the present study were performed 1/2—10 years (mean 3 years and 5 months) after the acute episode. Three important points should be discussed if the results of this study are to be compared with those of other investigators:

1. The type of exercise test used.
2. Criteria used to designate a pathological electrocardiographic response.
3. The selection of the material.

1. Type of exercise test

It seems reasonable to assume that the number of pathological ECG responses to exercise tests will be to some extent dependent upon the degree of energy expenditure during an exercise test. The energy expenditure during bicycle ergometry has been shown to be fairly constant when comparing normal subjects in a steady state. At a work load of 300 kpm/min the oxygen consumption is 1.0 l/min and at 600 kpm/min 1.6 l/min. These figures permit a rough comparison with Ford and Hellerstein's (3) studies with the single Master two-step test. They cal-

culated an average work load of 480 kpm/min (work against gravity) and an oxygen consumption during the test of 1 0 l/min. In their study the subjects never reached a steady state and the total energy cost of the test was calculated from the excess oxygen consumption during both the test and recovery periods. The total net oxygen consumption per minute exercise calculated in this way amounted to 1.5 l/min. During the double Master step test the subjects might be expected to reach this level of energy expenditure It can thus be concluded that patients working on the bicycle ergometer at 600 kpm/min have performed an exercise test at an energy expenditure of the same order of magnitude as the double Master step test In the present study 84 % of the men were tested at this level (600 kpm/min) but only 50 % of these reached a steady state.

2 Electrocardiographic criteria

The criteria of abnormality employed in this study are in general agreement with those discussed by Lepeschkin (7) in his review of exercise tests for the diagnosis of coronary heart disease It is generally agreed that a plateau depression (ischaemic S-T depression) should be considered as a pathological response (7) An isolated S-T junction depression was disregarded in this study This is in accordance with Robb et al (11) Such changes have also been found among 6—25 % of apparently normal persons (7)

3 Selection of cases

Comments on results

All cases with markedly pathological electrocardiograms at rest and/or with recent attacks of angina pectoris or a second infarction were excluded from the exercise test. The results must be considered with due regard to this selection.

The incidence of only 35 % of cases with an abnormal electrocardiographic response on exercise may be considered to be a small number in patients with definite evidence of past myocardial infarction.

Ford and Hellerstein (3) found 40 % abnormal ECG responses after a single Master test using Master's criteria in a group comparable to ours. Russek (12) used the double Master two-step test in a group of patients with angina pectoris and/or a known myocardial infarction and found 61 3 % with plateau depressions. An abnormal exercise tolerance test is generally regarded as evidence o coronary insufficiency (10) A normal electrocardiographic response to exercise on the other hand does not rule out the possibility of coronary disease and the existence of a previous healed myocardial infarction.

The incidence of 55 % of normal exercise tests in our material is higher than that reported by Smith and Lamb (14) who briefly stated it to be 30 % in cases of healed myocardial infarction.

This discrepancy may be explained by other principles of selection.

Uncharacteristic electrocardiographic responses

These electrocardiographic changes on exercise are difficult to explain. It may be stated that the observed alterations most certainly were not correlated with changes of the anatomical position of the heart because the changes were nearly always (except in 3 cases) observed also after the exercise test in the supine position. This finding also rules out the possibility of altered lead position because the changes were observed both with

H- and R-leads. However there was no predominance of angina pectoris in the group of patients with these "labile" changes of repolarization. The exact nature of the related alterations still remains obscure but we do not regard them as signs of severe ischaemia like those seen in acute myocardial infarction. The altered S-T T pattern was correlated with an increased heart rate.

Angina pectoris

In part II of this study (9) we reported that there was no significant correlation between the incidence of a history of angina pectoris and the degree of pathological alterations of the electrocardiogram at rest. It might be expected that the result of an exercise tolerance test would yield a better correlation between these two abnormalities. This, however does not apply to the present investigation. In this connection it is to be noted that the patients who were accepted for the exercise test certainly represent a selection of cases with a minor degree of coronary insufficiency In the group of patients with angina pectoris 45 % were excluded because of a history of recent attacks of severe angina pectoris, of second myocardial infarction, or of marked changes of the electrocardiogram at rest. Only 25 % of the cases in the groups of patients without chest pain had to be excluded If all patients had been submitted to the exercise test there might have been a better correlation between positive tests and the incidence of angina pectoris as judged from the case histories. On the other hand there was a much better correlation between the incidence of chest pain during the performance of the exercise test and the electrocardiographic response.

Physical working capacity

The heart rate during muscular work is highly correlated with the degree of exercise performed, rising linearly with increasing work loads. Furthermore, there is a correlation between heart-rate values in the steady state and the individual's oxygen uptake (Sjöstrand (13) Åstrand, (15) and others) Thus the heart-rate values obtained during the exercise test at "submaximal" work loads may serve as a rough guide for the evaluation of the patient's working capacity or physical fitness. Mean heart-rate values in the present series have been thus used in comparison with the corresponding values for a control group

When considering the physical working capacity of our patients two distinct categories may be recognized

1) Fourty-one cases who reached "steady state" at 600 kpm/min or more seemed to have ordinary physical working capacity with lower heart rate at corresponding work loads than the controls. It seems, however unlikely that the cases with myocardial infarction who reached 600 or 900 kpm/min at steady state should have a better working capacity than normal even if they had a significantly lower heart rate at corresponding work loads.

2) Twenty-six cases reached a "steady state" at 300 kpm/min only They had an average heart rate which was significantly higher than in the first group. The physical working capacity of these cases may therefore be lower

In 27 cases the working capacity was presumably restricted as a result of cardiac disease since the test had to be discontinued because of chest pain or an abnormal electrocardiographic response or because the patient did not reach a steady state at the lowest work load.

Conclusions regarding the patient's physical working capacity should be interpreted cautiously because all tests have been performed at submaximal work loads. In most cases it would have been desirable to continue the exercise with increasing loads and consequently higher heart rates. For obvious reasons, however the exercise tests have been performed with special caution in view of the previous myocardial infarction.

Summary

The present investigation deals with the results of exercise tolerance tests performed in a group of 94 cases among 155 re-investigated cases of past myocardial infarction which occurred below the age of 55 years. Sixty-one patients were excluded from performing the exercise test on account of repeated myocardial infarctions or markedly pathological electrocardiographic tracings at rest or because they refused the test. The following general conclusions may be made

1) Thirty five per cent of the cases tested had a pathological electrocardiographic response to graded muscular work. There was no correlation between the ECG changes during the acute episode and the ECG reaction during work at the re-examination. There were more cases with a normal ECG reaction during work among those with a normalized ECG than among the patients with a pathological ECG at re-examination.

2) An abnormal electrocardiographic response did not parallel the medical history of angina pectoris. However there was a fairly good correlation between the incidence of chest pain during the test and an abnormal electrocardiographic response.

3) Nearly 50 % of the cases tested seemed to have an ordinary working capacity as judged from heart rate values during graded muscular work on a bicycle-ergometer

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Clinical and Hemodynamic Assessment of Mitral Stenosis and Insufficiency

By

O. Storstein, R. Rokseth and L. Efskind

Accurate diagnosis of mitral valve disease is sometimes difficult. In particular when stenosis and insufficiency occur together the assessment of relative severity may be subject to error. The practical surgical implication is important. There is a diversity of opinion concerning the value of various signs indicating significant insufficiency. Some authors (e. g. Belgden and Leatham (1), Nixon and Wooler (9)) stress the importance of radiological and clinical signs. Others, as Dutrey and Drake (2) find hemodynamic results superior to clinical diagnosis. Owen and Wood (10) as well as Morrow et al. (8) studied pulmonary wedge pressure tracings and left atrial curves and suggested indices derived from the rate of y descent for the recognition of insufficiency. Marshall et al. (7) found hemodynamic measurements valuable, but no method could completely separate patients with predominant stenosis from those with predominant insufficiency. Venner and Holling (15) found wedge pressure tracings of little value, and Hamer et al. (5) concluded that no estimate of relative stenosis and insufficiency can be expected from the form of the pressure pulse without knowledge of the left atrial volume-pressure curve.

Because of diverging views we found it necessary to form our own opinion and present in this paper clinical and hemodynamic studies in 100 consecutive patients who were referred to operation for mitral stenosis.

Methods

Our study comprises all patients who in the course of two years were thought to have operable mitral stenosis. All were studied clinically by us. A twelve lead electrocardiogram was always recorded. Hemodynamic measurements, including right or left heart catheterization, was the routine in our laboratory except when clear-cut signs of severe, pure mitral insufficiency were present. Dye dilution methods or angiocardiography have not been used because of practical and theoretical objections. It was attempted to obtain pulmonary wedge tracings or left atrial pressure curves. This was successful in 84 patients. Hemodynamic data were incomplete

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Table IV ECG in 100 patients with mitral stenosis

Normal	18
Abnormal P-wave	12
Auricular fibrillation	38
Auricular flutter	5
Right ventricular hypertrophy	33
Left ventricular hypertrophy	11

siderable insufficiency the murmur was weaker grade 2, but a phonocardiogram confirmed the murmur to be pansystolic.

Electrocardiogram

Left ventricular hypertrophy was never found in patients with slight or no insufficiency but was present only in about a third of those with considerable insufficiency As seen in table III and fig 1 the presence of right ventricular hypertrophy was more related to pulmonary artery pressure than to valve lesion.

Table IV shows the electrocardiographic findings in the whole material. Auricular fibrillation was found in 38 patients and was not related to pulmonary artery pressure, but, as generally recognized, was found more often in the older age group. The mean cardiac index was 2.3 as compared to 3.2 in patients with normal sinus rhythm. P-wave changes alone were, with one exception, not found in considerable insufficiency

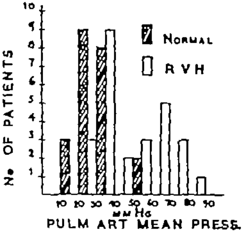


Fig. 1 ECG in mitral stenosis in relation to pulmonary artery mean pressure.

Radiological findings

As shown in table III left ventricular enlargement was found in about 2/3 of the patients with considerable insufficiency Insufficiency was suspected in

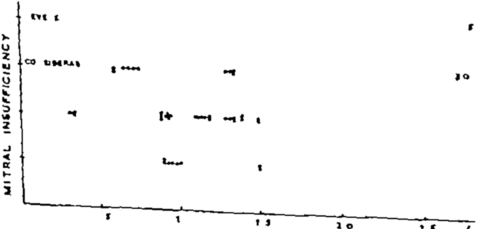


Fig 2. Relationship between calculated R V/V ratio and surgeons estimate of mitral insufficiency

Table I Material (23 men and 77 women)

Age (years)	
20–30	3
30–40	31
40–50	43
50–60	23
Total	100

Table II Clinical findings in 90 patients with mitral valve disease

Apical systolic murmur grade 3	17
Considerable mitral insufficiency	12
Slight mitral insufficiency	5
Mitral stenosis	0
Considerable mitral insufficiency	30
Apical systolic murmur absent	5
Apical systolic murmur less than grade 3	13
Pansystolic murmur grade 2	5
Apex displaced to the left	26
Aortic valve disease	6
Mitral insufficiency	12
Mitral stenosis	8

in 11 and 5 were referred to operation on clinical data alone.

The material is presented in table I Mitral valve disease was proved in all patients. A few died before operation and the diagnosis was then confirmed at autopsy Some patients had a low blood pressure during operation, which made the assessment of regurgitation doubtful It was possible to compare the preoperative data with the findings at operation in 90 patients.

Systolic murmurs were classified according to Levine. The criteria for left and right ventricular hypertrophy as judged from the electrocardiogram, were those of Sokolow and Lyon (13, 14) and Rasmussen (12) R y/V ratio was calculated according to Owen and Wood (10)

Results

Mitral stenosis was found in all patients. In 2, however the stenosis was minimal and they may be regarded as having pure" mitral insufficiency Twenty-eight patients had in addition to stenosis considerable insufficiency "Considerable" indicates in this study a strong regurgitant jet, as estimated by the surgeon (Leif Efskind M. D) regardless of the severity of accompanying stenosis. Insufficiency was slight to moderate in 42 patients, and completely absent in 18.

Table III ECG X-ray and R y/V related to mitral insufficiency

	Mitral insufficiency		
	Considerable	Slight	Absent
ECG			
Left ventricular hypertrophy	8	0	0
Right ventricular hypertrophy	10	15	5
Combined ventricular hypertrophy	1	2	0
X-ray			
Left ventricular enlargement	19	7	2
Giant left atrium	2	1	0
R y/V			
More than 1.6	6	4	0
1 6 or less	16	33	13
Total no. of patients	30	42	18

Clinical findings

As seen in table II the apex impulse was felt to the left of the midclavicular line in 26 patients. Eight of these had slight or no insufficiency A lifting apical impulse was present only in considerable insufficiency but was not a constant sign. The finding of an opening snap was of little value in accurate diagnosis. A pansystolic murmur grade 3 or more, was noted in 17 patients. All of these had mitral insufficiency which in 12 was considerable. In 5 other patients with con-

between R y/V ratio and surgical findings was possible in 72 cases. The average ratio was 1 45 in those with considerable insufficiency and 1 10 in those with slight or no insufficiency But fig. 2 shows that there was a great deal of overlapping, and no clear distinction is possible. A ratio over 1.6 however was always accompanied by insufficiency as noted in 10 patients, although insufficiency was slight to moderate only in 4 In somewhat less than 50 %, the calculated valve area, according to Gorlin and Gorlin (4), *correlated* well with the surgeon's estimates.

The pulmonary wedge or left atrial mean pressure in relation to pulmonary artery mean pressure is seen in fig 3 Fig 4 shows pulmonary artery mean pressure related to calculated pulmonary vascular resistance. Various degrees of raised pulmonary artery pressure and resistance may obviously occur with only moderately raised left atrial pressure. One extreme case had a pulmonary artery mean pressure of 70 mm Hg associated with a left atrial mean pressure of 15 mm Hg

Valve calcification

In table V mitral valve calcification is related to degree of mitral insufficiency No attempt has been made to grade cases with moderate calcification, which are excluded. Although heavy calcification was often accompanied by considerable insufficiency in more than half of the cases, the insufficiency was slight to moderate or absent.

Table V Calcification of mitral valves

Calcification	Mitral insufficiency		
	Considerable	Slight	Absent
Absent	3	15	11
Heavy	8	9	2

Comments

The assessment of mitral regurgitation during altered hemodynamics at operation is probably not infallible. A strong regurgitant "jet" moreover may not always signify a large regurgitant volume. An example of this is a patient who preoperatively was thought to have dominant mitral stenosis with little or no regurgitation. At operation the regurgitation was estimated to be severe, and the stenosis less pronounced. After a "small" valvulotomy however clinical examination showed subjective and objective improvement. Long term follow-up may as suggested by Lowther and Turner (6) add to accuracy in judgement.

With these reservations in mind it is apparent from our study that the assessment of mitral insufficiency in the presence of stenosis may be erroneous despite complete clinical and hemodynamic examination. In 7 patients there was no preoperative evidence of the considerable insufficiency found at operation. One patient had all clinical signs of severe, pure mitral stenosis confirmed by a very large left atrium — left ventricular diastolic pressure gradient. At operation a tight stenosis as well as a considerable regurgitant jet was found. Our findings are in accordance with the suggestions made by Wade et al. (16) that mitral incompetence may occur without the classical signs and that a severely narrowed mitral valve is also often incompetent.

With clinical examination, electrocardiogram, phonocardiogram and X-ray of the heart considerable insufficiency

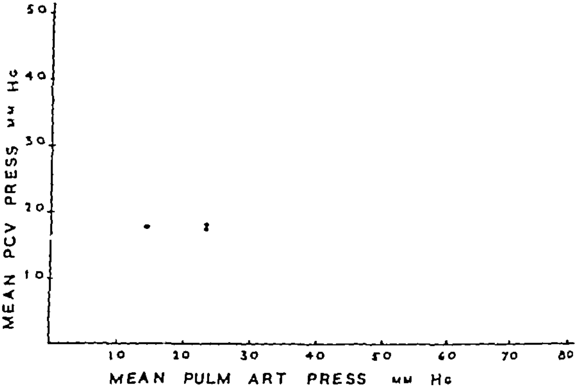


Fig 3. Relationship between pulmonary artery and pulmonary capillary venous pressures in mitral stenosis.

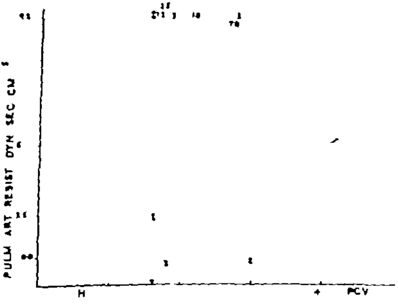


Fig 4. Relationship between pulmonary capillary venous pressure and pulmonary arteriolar resistance

2 more patients because of a giant left atrium. On the other hand a slight left ventricular enlargement was thought to be present in 2 patients where no insufficiency was noted at operation.

Hemodynamic examination

In some cases the pulmonary wedge tracings and the left atrial curves could not be satisfactorily analyzed because of tachycardia or damping Comparison

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could be predicted in more than 60 %. An elevated R y/V ratio calculated from the pressure curves, was less consistent. In 2 patients however it was the only sign of significant regurgitation. Some observers state that the left atrial pulse curve gives more accurate information than the pulmonary wedge tracing. In 31 of our patients left atrial curves were obtained but the R y/V ratio showed the same degree of overlapping as with wedge tracings. Our base line for pressure measurements was the anterior axillary line. Adjustments of the pressures to the sternal angle as referred to by Owen and Wood (10) does not give a clearer distinction between degrees of insufficiency.

The electrocardiographic finding of right ventricular hypertrophy in 10 patients with considerable mitral insufficiency may seem surprising. In 30 patients with pure insufficiency Brigden and Leatham (1) found none with right ventricular hypertrophy. Friedberg (3) states that the presence of right ventricular hypertrophy strongly supports a diagnosis of pure or predominant mitral stenosis. On the other hand Rapaport and Richman (11) noted right ventricular hypertrophy either isolated or as combined hypertrophy in 10 patients with predominant insufficiency. It is reasonable to believe, as our study seems to indicate that pulmonary artery pressure is the determinant factor.

Summary

Clinical and hemodynamic studies have been made in 100 consecutive patients who were referred to operation for mitral stenosis. The preoperative findings were compared with the surgeon's estimates. A significant regurgitant "jet" was found in 30 patients. This could be expected from clinical electrocardiographic, phonocardiographic and radiological findings in about 2/3 of cases. An R y/V ratio of more than 1.6 was found in 10 patients and all of them had insufficiency which in 6 was considerable.

It is concluded that the diagnosis of mitral insufficiency in the presence of stenosis was sometimes impossible as there were no preoperative signs to indicate considerable insufficiency found in 7 patients. Clinical examination, including electrocardiogram, phonocardiogram and radiogram was most valuable, but in 2 cases an elevated R y/V ratio was the only sign of considerable mitral insufficiency.

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From the Second University Clinic of Internal Medicine (Head K. Lundbæk, M. D.)
Kommunehospitalet, Aarhus, Denmark

The Insulin-like Activity in Serum Determined by the Rat Epididymal Fat Method

III. Insulin Antagonism in Serum from Normal Persons and Diabetics, Including some Observations on Pig's Blood

By

Jens Lyngsøe

In a number of studies in which the insulin-like activity in serum (SILA) has been determined by either the rat diafragm or the rat epididymal fat method the effectiveness of these methods has been tested by adding insulin to the serum. In the majority of such studies the insulin added to serum from normal persons has been recovered quantitatively (17, 25, 30).

Testing sera from diabetic subjects by means of the rat diafragm method the insulin recovery from some of the sera was found to be less than in normal subjects (8, 14, 31, 36). This phenomenon was explained by the presence in the serum of factors which inhibited the action of insulin on the rat diafragm. Such factors were called insulin antagonists, a designation subsequently applied to a number of substances inhibiting the action of insulin when given to animals or used in experiments with isolated tissue (3, 33). In the present study the term insulin antagonist has been used only to designate a substance inhibiting the action of insulin on isolated tissue in vitro.

In the studies published on the insulin antagonism of serum large amounts of insulin were added to the serum, varying from 250 μU/ml to 200 000 μU/ml.

In the present study an account will be given of some investigations on insulin recovery and insulin antagonism. These investigations were made with the rat epididymal fat method, using undiluted and diluted serum from normal subjects and from diabetics. Some studies were also made on pig's serum. In contrast to the procedure followed by the investigators mentioned above, only a small amount of insulin was added to the sera, 100 μU/ml, in the hope that thereby a possible weak antagonism of insulin might be revealed.

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Table I. Clinical data on patients with diabetes mellitus

Pt. no.	Sex	Age	Age at onset	Ht. cm	Wt. kg	Ideal wt.	Wt. %	Insulin I. U./24 hrs.	Duration of insulin treatment (yrs)	Last dose of insulin before blood sampling I. U	Blood sugar on day of examination 0700, 1500, 1700	Urine after exam.	24-hour urine on day of examination	Total CO_2 mEq/l
223	M	23	7	177	67	68.8	<100	42	16	42 I. U 48 hrs before exam.	423–410 184	3.1 % +G+L	50 g +G+L	7
226	F	61	52	159	75	60.5	124	52	—	—	309–247– 187	4.4 " +G+L	—	6
227	M	27	6	173	63	74.3	<100	60	21	24 I U 24 hrs before exam.	265–261– 249	2.4 % +G+L	55 g +G+L	—
228	M	59	35	181	68	80.4	<100	12	24	12 NPH 24 hrs before exam.	235–217– 179	—	22 g −G−L	—
229	F	44	28	165	60	65.1	<100	32	16	12 NPH 36 hrs before exam.	363–321– 247	1.4 +G+L	55 g +G+L	10
231	M	23	23	192	65	85.5	<100	24	—	—	212–406– 314	1.8 % −G+L	76 g −G+L	31
232	M	15	15	178	44	—	—	52	—	—	406–198– 160	2.4 +G+L	20 g +G+L	14
233	M	71	71	163	74	68.5	100	—	—	—	186–193– 156	—	8 g −G−L	—
234	F	42	40	148	44	52.6	<100	132	2	36 NPH + 12, 24 hrs before exam.	186–364– 364	1.0 % −G−L	20 g −G−L	—
II.								224		118 NPH + 56, 24 hrs before exam.	55–216– 222	0.7 % −G−L	8 g −G−L	
III.								88		12 NPH 14 hrs befor exam.	47—	−G−L	—	—
235	M	53	53	167	66	68.8	<100	32	—	—	256–391 234	2.5 % −G−L	62 g −G−L	—
236	F	58	58	162	93	62.8	156	12	—	—	187–264– 216	1.7 " −G−L	10 g −G−L	—
237	M	71	71	169	86	71.3	121	Tolbutamide	—	—	160–218– 163	1.9 " −G−L	35 g −G−L	—

Material

The normal subjects studied were patients hospitalized for diseases unaccompanied by glycosuria or elevated blood sugar. None of the normal subjects had symptoms of diabetes mellitus. None had glycosuria on examination of the 24-hour urine with Clinistix, and none had fasting glucose levels higher than 100 mg % on examination of the serum used for the SILA determination.

The series of diabetics consist of both those newly diagnosed and those with a longer history of diabetes (table I). None of the patients were icteric. The patients with diabetic acidosis all had ketonuria, and in 4 out of 5 patients the total blood CO_2 was below 15 mMol/l. The total CO_2 was not determined in the fifth patient, but as the patient complained of nausea, had slight polypnea, slightly reduced skin turgor, ketonuria and polyuria, acidosis was probably present here too. One of the patients with acidosis was unconscious at the time the blood sample was taken for the SILA determination (patient no. 206), patient no. 183 was somnolent, while the remaining patients were fully conscious.

Only one of the patients who were not acidotic had ketonuria (no. 251). The total blood CO_2 was 31 mMol/l. Patient no. 219 had undergone hypophysectomy 9 months before the examination.

One of the diabetics (no. 254) had a remarkably high insulin requirement. During one period, the daily insulin dose was more than 200 I.U. The blood sugar values in the same period lay between 100 and 200 mg %. In this patient, blood was taken for SILA determinations on three occasions when the values of the daily insulin dose differed.

Three of the patients had active acromegaly but a normal glucose tolerance test (nos. 189, 271 and 231). One acromegalic patient had diabetes.

Patient no. 270. Man aged 52. Has three maternal cousins with diabetes mellitus. Since the age of 40, there has been growth of the hands, feet, lower jaw, lips and ears. Irradiation of the hypophysis by X-rays at the ages of 43 and 41. Growth of the soft tissues then ceased until the age of 50 when the patient again noticed growth of the fingers. Six months before the examination he underwent irradiation of the pituitary with Co[60].

Oral glucose tolerance tests gave normal results at the ages of 41, 43 and 45. Glycosuria was observed six months before the present investigation and a glucose tolerance test at that time showed diabetes. Polydipsia and polyuria developed subsequently.

The patient had a typical acromegalic appearance, with enlarged lower jaw, broad lips, large ears and nose, macroglossia and lumbar kyfosis. The hands and feet were large and plump. Ophthalmoscopy showed no diabetic retinal changes. Perimetry was normal. A roentgenogram of the sella turcica showed enlargement to 24 × 18 mm, PBI was 5.4 µg %, basal metabolic rate was 121 and 119 %. Excretion of pituitary gonadotropins in the urine was normal. Fractionated 17-ketosteroid determination (18) showed 25.4 mg/24 hours, "aetiocholanon fraction and rest-fraction" being increased. Satisfactory regulation of the diabetes was achieved by diet and NPH insulin 16 I.U. a day.

Procedure and methods

Pig serum was obtained from porkers. An ear vein was bled by incision 1—3 hours after feeding, after thoroughly washing the ear with alcohol. The blood was immediately placed in a water bath at 0° C. After standing for 1—2 hours the blood was centrifuged at 4 000 rpm for 10 min. at room temperature. The clot was loosened by a swab stick, and the blood centrifuged again at room temperature, 4 000 rpm for 5 min. The serum was pipetted off and stored at 4° C.

Normal subjects and patients were hospitalized during the investigation. The insulin-requiring diabetics had not received insulin more recently than 24 hours before examination, with the exception of patients no. 254 and 261, who received NPH insulin 14 hours before taking blood samples.

Human blood for SILA determination was taken from an arm vein, applying slight stasis, between 7 and 8 a.m. after fasting and bed-rest for 8—12 hours. Patient no. 254 was however examined once while ambulant, after having been up for one hour.

Serum for determination of SILA was prepared from human venous blood as described previously (23). SILA was determined in both

Table I. Clinical data on patients with diabetes mellitus

Pt. no.	Sex	Age	Age at onset	Ht. cm	Wt. kg	Ideal wt.	Wt. %	Insulin I.U./24 hrs.	Duration of insulin treatment (yrs)	Last dose of insulin before blood sampling I.U	Blood sugar on day of examination 0700, 1300, 1700	Urin after exam.	24-hour urine on day of examination	Total CO mEq/l
183	M	23	7	177	67	68.8	<100	42	16	42 I.U. 48 hrs before exam.	423–410 184	3.1 % +G+L	50 g +G+L	7
206	F	61	52	159	73	60.5	124	52	–	–	300–247–187	4.4 % +G+L	–	6
217	M	27	6	173	63	74.3	<100	60	21	24 I. U. 24 hrs before exam.	265–261 249	2.4 % +G+L	55 g +G+L	–
219	M	59	35	181	66	80.4	<100	12	24	12 NPH 24 hrs before exam.	235–217–179	–	22 g –G–L	–
225	F	44	28	165	60	63.1	<100	32	16	12 NPH 96 hrs before exam.	365–321–247	1.4 % +G+L	33 g +G+L	10
231	M	23	23	182	63	83.5	<100	24	–	–	212–408–314	1.8 % –G+L	76 g –G+L	31
232	M	15	15	170	44	–	–	52	–	–	406–198–160	2.4 % +G+L	20 g +G+L	14
233	M	71	71	165	74	68.5	108	–	–	–	186–193–156	–	8 g –G–L	–
234	F	47	40	148	44	52.6	<100	132	2	36 NPH + 12, 24 hrs before exam.	186–364–364	1.0 % –G–L	20 g –G–L	–
II								224		118 NPH + 56, 24 hrs before exam.	55–216–222	0.7 % –G–L	8 g –G–L	–
III								88		12 NPH 14 hrs before exam.	47–	–G L	–	–
235	M	53	53	167	66	68.8	<100	32	–	–	236–391 254	2.3 % –G–L	62 g –G–L	–
236	F	58	58	162	98	62.8	156	12	–	–	187–264–216	1.7 % –G–L	10 g –G–L	–
237	M	71	71	169	86	71.3	121	Tolbutamide	–	–	160–218–165	1.9 % –G–L	35 g –C–L	–

Table I (cont.)

Pt. no.	Sex	Age	Age at onset	Ht. cm	Wt kg	Ideal wt.	Wt. %	Insulin I U/24 hrs.	Duration of insulin treatment (yrs)	Last dose of insulin before blood sampling I U	Blood sugar on day of examination 0700, 1300, 1700	Urine after exam.	24-hour urine on day of examination	Total CO mEq/l
258	M	57	57	159	83	63.8	130	–	–	–	213–224–313	3.5 % –G–L	65 g +G+L	–
259	F	57	57	162	69	62.8	109	20	–	–	241–412–325	3.3 „ –G–L	10 g –G–L	–
260	F	79	70	150	69	53.9	128	32	9	32 NPH 24 hrs before exam.	153–194–145	0.3 „ –G–L	3.6 g –G–L	–
261	F	54	24	156	52	58.3	<100	48	30	16 NPH 14 hrs before exam.	134–256–111	– –G–L	0 g –G–L	–
262	F	56	56	168	78	67.3	116	Tolbutamide	–	–	223–227–235	6 % –G–L	37 g –G–L	–
263	M	28	28	167	57	68.8	<100	36	–	–	249–402–426	–	135 g –G–L	–
264	F	31	31	169	59	68.1	<100	–	–	–	181–244–228	2.1 „ –G–L	53 g –G–L	–
265	M	15	15	172	55	–	–	12	–	–	230–258–224	2.5 % –G–L	34 g –G–L	–
266	F	22	2	155	48	57.3	<100	44	20	24 NPH 24 hrs before exam.	195–238–218	1.2 % –G–L	37 g –G–L	–
267	F	25	11	163	62	63.6	<100	76	14	36 NPH 24 hrs before ex.	269–401–413	–	120 g –G–L	–
268	M	67	67	169	79	71.3	111	32	–	–	279–437–341	–	200 g –G–L	–
269	F	47	47	167	50	66.6	<100	20	–	–	322–400–336	3.8 % –G–L	76 g –G–L	–
270	M	52	51	186	106	84.1	126	16	–	–	185–183–189	–	6 g –G–L	–

undiluted serum and serum diluted 1/5 with Krebs-Ringer-bicarbonate buffer, a modification of the rat epididymal fat method being used (22). SILA values less than 10 μU/ml were reported as 0 μU/ml. The tables and calculations in the present report include only determinations with an index of precision less than 0.34.

Insulin was added by pipetting 10 μl of an insulin solution of 20,000 μU/ml into each incubation tube containing 2 ml serum or serum dilution. Care was taken on adding the in-

sulin solution to avoid air bubbles in the serum. The insulin used was prepared from pig pancreas and contained 27 1 μU/ml.

The albumin fraction containing the insulin antagonist described by Vallance-Owen, was prepared as indicated by this worker (31) After precipitation with 10 % trichloroacetic acid-ethanol (TCA-ethanol) the precipitate was washed with 5 % TCA-ethanol, and the albumin then dissolved in 1 % TCA-ethanol. After centrifugation the supernatant was dialysed against water for 24 hours. The pH was then 7.3—8.3. Prior to freeze-drying, the solution was concentrated to 1/2—1/3 the volume at 30° C under low pressure produced by a water suction pump. This caused weak formation of foam. The concentrated solution containing the serum albumin was then freeze-dried. Prior to incubating, the freeze-dried albumin was dissolved in Krebs-Ringer bicarbonate buffer in an amount corresponding to the original serum volume. T examine the antagonistic effect of the albumin, experiments were performed in which pieces of epididymal fat were incubated in the buffer with 100 μU insulin per ml, while other pieces ere incubated in the albumin solution described above, to which 100 μU/ml had been added. The amount of $C^{14}O$ formed in the incubation tubes, which is measure of the action of the insulin, was calculated as

$$Ak = CpM \times \frac{10}{\text{fat weight}}$$

Δ Ak was calculated by subtracting the Ak values in the incubation tubes without albumin from the Ak values in the tubes with albumin. A negative Δ Ak indicates an inhibition of the insulin in the albumin solution, i. e. an antagonistic effect.

The serum proteins from one of the patients (no. 251) were fractionated by electrophoresis in a polyvinylchloride block (6) The block was moistened with barbiturate buffer at pH 8 6, μ 0.1 The electrophoresis was done at 4° C with a current strength of 18—21 mA. After continuing the electrophoresis for 24 hours, the protein fractions were localized in the polyvinylchloride block as follows: a strip of filter paper was placed the length of the block and dipping 2—3 mm into it. In this way a little liquid was sucked up by the paper which was then stained with bromphenol blue. The albumin, $α_1$- β- and γ-globulins could now be clearly distinguished visually on the paper strip. From the localization of the protein fractions on the paper it was possible to measure the various localizations in the block. This was now cut into 8 sections corresponding to albumin, inter albumin-$α_1$-globulin, $α_2$-globulin, inter $α_2$-β-globulin, β-globulin, inter-β-γ-globulin, and a rapid and slow γ-globulin fraction. Each protein fraction was eluted from the block with 0.9 % NaCl, dialysed for 24 hours against water at 4 C, freeze-dried from the frozen state and then redissolved in a Krebs-Ringer-bicarbonate buffer the volume of which was between 1 and 9 times that of the serum volume used for the electrophoresis.

The glucose concentration in serum for SILA determination was measured by the glucose oxidase method (16) The blood sugar determinations to indicate the 24-hour level were made by the Hagedorn-Norman Jensen method (15) The total CO in the blood was measured by van Slyke' method (27) The urine sugar level was determined by reduction method, using potassium ferricyanide.

Results

Tables II—VIII show the results obtained on examining blood from normal subjects, pigs, diabetics presenting diverse clinical characteristics, and a small number of patients with acromegaly

It is seen in table II that when 100 μU of pig insulin per ml was added to *human serum*, there was a rise in SILA which, in undiluted serum, corresponded to the amount of insulin added on the average 93 μU/ml. On adding the insulin to *diluted human serum*, the average rise in SILA was greater 125.2 μU/ml. On statistical analysis this figure is not different from 100.

In the experiments in which pig insulin was added to *pig serum* (table II)

[1] The insulin was supplied through the courtesy of Dr H. G. Hagedorn, M. D Nordisk Insulin Laboratorium.

Pevikon C 870, Fosfatbolaget, Stockholm, Sweden.

Table I (cont.)

Pt. no.	Sex	Age	Age at on-set	Ht cm	Wt kg	Ideal wt	Wt. %	Insulin I. U /24 hrs.	Duration of insulin treatment (yrs)	Last dose of insulin before blood sampling I. U	Blood sugar on day of examination 0700, 1300 1700	Urine after exam.	24-hour urine on day of examination	Total CO mEq/l
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269	F	47	47	167	50	66.6	<100	20	–	–	322–400–336	3.8 % –G–L	76 g –G–L	–
270	M	5	51	186	106	84 1	126	16	–	–	185–183–189	–	6 g –G–L	–

undiluted serum and serum diluted 1/3 with Krebs-Ringer bicarbonate buffer a modification of the rat epididymal fat method being used (22) SILA values less than 10 μU/ml were reported as 0 μU/ml. The tables and calculations in the present report include only determinations with an index of precision less than 0.34

Insulin was added by pipetting 10 μl of an insulin solution of 20,000 μU/ml into each incubation tube containing 2 ml serum or serum dilution. Care was taken on adding the in-

Table III. Insulin addition to serum from non-acidotic diabetics

Serum no.	Pt. no.	Serum glucose mg%	SILA Undiluted serum μU/ml	Serum + 100 μU/ml	SILA Diluted serum μU/ml	Diluted serum + 100 μU/ml	No. of rats	Index of precision
Untreated juvenile diabetics								
630	263	246	36	95	15	60	4	0.23
695	264	185	108	280	16	260	4	0.20
714	265	81	0	97	0	54	3	0.22
538	231	208	175	285	44	200	4	0.24
Mean			80	189	19	144		
Untreated old non-obese diabetics								
596	233	199	180	340	55	310	4	0.21
592	255	263	82	215	30	220	3	0.24
606	269	317	160	440	57	440	4	0.18
614	259	266	87	215	28	160	3	0.29
694	268	296	40	180	67	235	3	0.33
Mean			110	278	47	273		
Untreated old obese diabetics								
599	256	192	88	240	24	190	4	0.21
607	257	176	103	590	22	190	4	0.17
613	258	209	560	680	95	580	3	0.18
612	262	213	320	600	195	660	4	0.21
Mean			268	528	84	405		
Insulin-treated diabetics								
617	219	234	42	170	23	105	4	0.29
620	261	142	240	350	32	130	4	0.29
641	266	195	163	300	92	225	4	0.20
671	267	232	195	580	46	350	3	0.33
615	260	176	103	278	19	172	4	0.22
Mean			153	336	46	192		

These values have not been multiplied by the dilution factor.

In table III these non-acidotic, untreated diabetics were divided into three groups: *juvenile* patients with diabetes mellitus diagnosed before the age of 40 years, *older non-adipose* diabetics with diabetes diagnosed after the age of 40 years and weighing less than 115 % of ideal weight (23) and *older adipose* diabetics, whose diabetes was diagnosed after the age of 40 years and whose weight exceeded 115 % of ideal weight. It appears that the mean insulin recovery in undiluted and diluted serum is about 100 μU/ml in the juvenile group but considerably over 100 μU/ml in the other two diabetic groups. Inhibition of the added insulin, or "insulin antagonism" was not demonstrated, therefore, in any

Table II Insulin addition to serum from normal individuals

Normal persons

Assay no.	No.	Serum glucose mg%	Insulin-like activity: Undiluted serum μU/ml	Insulin-like activity: + 100 μU/ml	Insulin-like activity: Diluted serum[1] μU/ml	Insulin-like activity: + 100 μU/ml	Index of precision	No. of rats
581	271	96	90	225	58	128	0.27	4
587	272	83	225	285	145	285	0.21	3
588	273	87	92	170	44	150	0.22	4
589	274	95	135	175	56	96	0.21	3
595	275	86	225	360	105	240	0.16	4
603	276	96	340	450	150	410	0.18	3
		Mean	181.5	277.5	93	218.2		
Insulin recovery mean				93		125.2		
Insulin recovery difference from 100 μU/ml				$t = 0.429$ $p > 0.1$		$t = 0.801$ $p > 0.1$		
Normal pigs								
568	7	77	155	310	320	450	0.15	3
569	8	120	150	280	64	285	0.18	3
570	9	135	125	230	48	290	0.23	4
571	10	109	17	135	27	165	0.31	4
572	11	99	128	200	51	185	0.18	3
		Mean	111	235	102	275		
Insulin recovery mean				124		173		
Insulin recovery difference from 100 μU/ml				$t = 1.702$ $p > 0.1$		$t = 3.107$ $p < 0.05$		

[1] These values have not been multiplied by the dilution factor.

a rise in SILA was found as in human serum. In *undiluted* serum the rise corresponded to the amount of insulin added but in *diluted pig serum* the rise showed a mean value of 173 μU/ml, which was *significantly greater than 100 μU/ml* ($p < 0.05$)

If the experiments entailing the addition of insulin to serum from *normal subjects* and *pig serum* are viewed as one group there is only a single instance in which the SILA values found are seen to derivate from the expected values by more than 25 % after adding 100 μU insulin per ml undiluted serum. In the experiments with diluted serum four out of the 11 sera show a SILA value deviating more than 25 % from the expected value and in both pig serum and human serum the mean increase in SILA is greater than 100 μU/ml, although significantly so only in the case of pig serum.

On adding insulin to serum from *non-acidotic untreated diabetics* more than the added 100 μU/ml was recovered in 11 out of 13 cases (table III) Corresponding results were found in diluted serum, the insulin amount recovered being higher than the amount added in the same 11 sera.

Table III. Insulin addition to serum from non-acidotic diabetics

Untreated juvenile diabetics

Ser. no.	Pt. no.	Serum glucose mg%	SILA Undiluted serum µU/ml	Serum + 100 µU/ml	SILA Diluted serum µU/ml	Diluted serum + 100 µU/ml	No. of rats	Index of precision
[illegible]	263	246	36	95	15	60	4	0.23
[illegible]	254	185	108	280	16	260	4	0.20
[illegible]	253	81	0	97	0	54	3	0.22
[illegible]	[illegible]	208	173	283	44	200	4	0.24
Mean			80	189	19	144		

Untreated old non-obese diabetics

Ser. no.	Pt. no.	Serum glucose mg%	SILA Undiluted serum µU/ml	Serum + 100 µU/ml	SILA Diluted serum µU/ml	Diluted serum + 100 µU/ml	No. of rats	Index of precision
[illegible]	231	199	180	340	55	310	4	0.21
[illegible]	233	[illegible]	82	215	30	220	3	0.24
[illegible]	[illegible]	317	160	440	57	440	4	0.18
[illegible]	[illegible]	[illegible]	87	215	28	160	3	0.29
[illegible]	258	[illegible]	40	180	67	235	3	0.33
Mean			110	278	47	273		

Untreated old obese diabetics

Ser. no.	Pt. no.	Serum glucose mg%	SILA Undiluted serum µU/ml	Serum + 100 µU/ml	SILA Diluted serum µU/ml	Diluted serum + 100 µU/ml	No. of rats	Index of precision
[illegible]	[illegible]	182	83	240	4	190	4	0.21
[illegible]	237	176	103	590	22	190	4	0.17
[illegible]	[illegible]	[illegible]	560	680	95	580	3	0.18
[illegible]	[illegible]	213	320	600	193	660	4	0.21
Mean			268	528	84	405		

Insulin-treated diabetics

Ser. no.	Pt. no.	Serum glucose mg%	SILA Undiluted serum µU/ml	Serum + 100 µU/ml	SILA Diluted serum µU/ml	Diluted serum + 100 µU/ml	No. of rats	Index of precision
[illegible]	219	234	62	170	23	105	4	0.29
[illegible]	251	142	240	350	52	130	4	0.29
[illegible]	256	195	165	500	82	225	4	0.20
[illegible]	[illegible]	222	193	580	46	330	3	0.33
[illegible]	[illegible]	176	183	278	19	172	4	0.22
Mean			153	336	46	192		

These values have not been multiplied by the dilution factor.

In table III these non-acidotic, untreated diabetics were divided into three groups: *juvenile* patients with diabetes mellitus diagnosed before the age of 40 years, *older non-adipose* diabetics with diabetes diagnosed after the age of 40 years and weighing less than 115 % of ideal weight (23) and *older adipose* diabetics, whose diabetes was diagnosed after the age of 40 years and whose weight exceeded 115 % of ideal weight. It appears that the mean insulin recovery in undiluted and diluted serum is about 100 µU/ml in the juvenile group, but considerably over 100 µU/ml in the other two diabetic groups. Inhibition of the added insulin, or "insulin antagonism" was not demonstrated, therefore, in any

Table II Insulin addition to serum from normal individuals

Normal persons

Assay no.	No.	Serum glucose mg%	Insulin-like activity: Undiluted serum µU/ml	Insulin-like activity: + 100 µU/ml	Insulin-like activity: Diluted serum[1] µU/ml	Insulin-like activity: + 100 µU/ml	Index of precision	No. of rats
581	271	96	90	225	58	128	0.27	4
587	272	83	225	285	145	285	0.21	3
588	273	87	92	170	44	150	0.22	4
589	274	95	135	175	56	96	0.21	3
595	275	86	225	360	105	240	0.16	4
603	276	96	340	450	150	410	0.18	3
		Mean	*184.5*	*277.5*	*93*	*218.2*		
Insulin recovery mean:				93		125.2		
Insulin recovery difference from 100 µU/ml				$t = 0.429$ $p > 0.1$		$t = 0.801$ $p > 0.1$		

Normal pigs

Assay no.	No.	Serum glucose mg%	Insulin-like activity: Undiluted serum µU/ml	Insulin-like activity: + 100 µU/ml	Insulin-like activity: Diluted serum[1] µU/ml	Insulin-like activity: + 100 µU/ml	Index of precision	No. of rats
568	7	77	155	310	320	450	0.15	3
569	8	120	130	280	64	285	0.18	3
570	9	135	125	230	48	290	0.23	4
571	10	109	17	155	27	165	0.31	4
572	11	99	128	200	51	185	0.18	3
		Mean	111	235	102	275		
Insulin recovery mean				124		173		
Insulin recovery difference from 100 µU/ml				$t = 1.702$ $p > 0.1$		$t = 3.107$ $p < 0.05$		

[1] These values have not been multiplied by the dilution factor

a rise in SILA was found as in human serum. In *undiluted* serum the rise corresponded to the amount of insulin added but in *diluted pig serum* the rise showed a mean value of 173 µU/ml which was significantly greater than 100 µU/ml ($p < 0.05$)

If the experiments entailing the addition of insulin to serum from *normal subjects* and *pig serum* are viewed as one group there is only a single instance in which the SILA values found are seen to derivate from the expected values by more than 25 % after adding 100 µU insulin per ml undiluted serum. In the experiments with diluted serum, four out of the 11 sera show a SILA value deviating more than 25 % from the expected value and in both pig serum and human serum the mean increase in SILA is greater than 100 µU/ml although significantly so only in the case of pig serum.

On adding insulin to serum from *non-acidotic untreated diabetics* more than the added 100 µU/ml was recovered in 11 out of 13 cases (table III) Corresponding results were found in diluted serum, the insulin amount recovered being higher than the amount added in the same 11 sera.

Table VI. Insulin antagonism in serum-protein fractions from a patient with insulin resistance

Fraction no.		Dilution	Assay no.	Index of precision	No. of rats	Insulin-like activity μU/ml	Insulin-like activity after addition of 100 μU/ml
1	Albumin	1/10	786	0.32	4	0	120
2	Albumin + α + α_1 globulin	1/1	777	0.28	4	70	205
3	α_1 globulin	1/7	786	0.32	4	20	175
4	α_1 + β globulin	1/1	777	0.28	4	540	340
5	β globulin	1/6	787	0.33	4	480	330
7	γ globulin	1/1	782	0.27	4	440	440
7	γ globulin	1/8	790	0.21	4	18	18
8	γ globulin	1/7	790	0.21	4	0	102

These values have not been multiplied by the dilution factor.

Table VII. Insulin addition to serum from acidotic diabetics

Assay no.	Patient no.	Serum glucose mg%	SILA Undiluted serum μU/ml	SILA Undiluted serum + 100 μU/ml	SILA Diluted serum μU/ml	SILA Diluted serum + 100 μU/ml	No. of rats	Index of precision
283	183	326	21	34	51	128	4	0.23
387	206	372	70	46	72	430	3	0.31
443	217	240	203	215	92	400	3	0.22
476	229	362	180	270	60	530	3	0.18
578	252	330	0	71	35	275	3	0.33
		Mean	95.2	127.2	62.0	352.6		
		Insulin recovery mean		32		290.6		
		Insulin recovery different from 100 μU/ml		t = 3.238, p < 0.05		= 2.887, p < 0.05		

These values have not been multiplied by the dilution factor

tion, expressed as Ak, was in three cases highest and in three cases lowest in the solution with albumin. In no case, however was the mean change in the activity significantly different from zero (table IV). The action of insulin on rat epididymal fat has thus not been inhibited by the albumin from these patients.

As stated, one of the diabetics examined was insulin resistant. The patient was examined on three separate occasions, I, II and III. On occasion II the patient showed insulin resistance, as the 24-hour dose of insulin exceeded 200 I.U. without signs of acidosis or infectious disease. An obvious inhibition of the added insulin was demonstrated at this stage, both in undiluted and diluted serum. At examinations I and III the patient showed no clinical insulin resistance, as the daily insulin dose was less than 200 I.U./24 hrs. In spite of this,

Table II The antagonistic effect of serum albumin from juvenile diabetics

Assay no.	Patient no.	CpM × 10 / ½ fat weight Buffer containing 100 μU/ml Mean ± standard error	Δ Ak Albumin solution containing 100 μU/ml Mean ± standard error	Albumin concentration %	Δ Ak, difference from zero		n
					t	p	
768	254	251 ± 29	44 ± 23	5.1	1.913	> 0.1	4
769	266	123 ± 26	13 ± 13	2.9	1.000	> 0.1	5
	267		−11 ± 14	4.3	0.786	> 0.1	5
	263		−30 ± 14	4.6	2.143	> 0.1	5
	265		−16 ± 16	4.6	1.000	> 0.1	5
772	264	246 ± 137	257 ± 137	2.3	1.876	> 0.1	4

Table V Insulin addition to serum from an insulin-resistant diabetic

Assay no.	Serum no.	Undiluted serum				Diluted serum				Index of precision	No. of rats
		SILA μU/ml	+ 100 μU/ml	+ 500 μU/ml	+1000 μU/ml	SILA μU/ml	+ 100 μU/ml	+ 500 μU/ml	+1000 μU/ml		
591	I	410	350	–	–	200	210	–	–	0.18	3
608	II	225	240	210	300	–	–	–	–	0.28	3
774	II	–	–	–	–	380	270	–	–	0.27	4
764	III	–	–	–	–	100	83	120	130	0.32	4

These values have not been multiplied by the dilution factor

of the groups of untreated non-acidotic diabetics examined. On the contrary it appeared that after adding insulin, the increase in SILA was 1 1/2 to 2 times greater than anticipated

Six *insulin-treated non-acidotic diabetics* were studied 24 hours after the insulin treatment had been suspended Of these, patient no 254 showed insulin resistance. Studies on this patient have been grouped separately After the addition of insulin to serum from the other insulin-treated diabetics, a greater increase in SILA was found on the average than corresponded to the added amount of insulin namely 183 and 156 μU/ml in undiluted and diluted sera respectively (table III) Insulin antagonism has thus not been demonstrated in these patients either but on the contrary after adding insulin to the serum, there was a rise in SILA of generally 1 1/2 to 2 times the amount anticipated

The *albumin fraction* was extracted from the serum of 6 *poorly regulated non-acidotic juvenile diabetics* as described by Vallance Owen et al. (31) The albumin was prepared from serum taken simultaneously with that used in the experiments on the addition of insulin. The albumin was dissolved in a Krebs-Ringer-bicarbonate buffer containing 100 μU insulin per ml and the $C^{14}O$ produced in this solution was compared with that formed in a corresponding solution without albumin. It appears that the average $C^{14}O$ forma-

In a previous study using the rat epididymal fat method, it was found that after adding insulin to normal serum, the rise in SILA was greater than corresponded to the amount of insulin added 100 μU/ml (21)

Other investigators report that using the rat epididymal fat method, the added insulin amount is recovered quantitatively (13 17 25) In the present study the experiments were carried out with a technic which deviated from that employed in the above-mentioned previous study In the earlier experiments when the insulin was added to the serum the pipette was emptied by blowing out. This blowing out which caused a weak formation of foam in the serum, was omitted in the present experiments. Using this new technic, it was shown, that when pig insulin is added to *undiluted serum* from *normal subjects* and *normal pigs*, the SILA increases correspond to the amount of insulin added. This difference between the first and subsequent experiments may reasonably be explained as the result of altering the technic used in adding the insulin.

In the present study when insulin was added to *diluted serum from pigs* a rise in SILA was found which was greater than the amount of insulin added. Likewise, in the experiments with *diluted human serum*, the average value of the insulin recovered was also greater than expected, although the rise was not statistically significant. Staub (28) also using the rat epididymal fat method has likewise found an increased insulin recovery on adding insulin to diluted serum.

The cause of the increased recovery of insulin from diluted serum is unknown. Some studies seem to show however that the SILA value in undiluted serum measured by the rat diafragm method and by the rat epididymal fat method constitutes only a part of the insulin amount present in the serum Part of the serum insulin appears to be bound to protein in an inactive form (1 2) It is conceivable that by adding insulin to diluted serum in which the normal equilibrium between free and bound insulin is presumably disturbed, this equilibrium is so displaced that endogenous insulin is released. The addition of insulin would result in the SILA value being increased by more than the amount of insulin added

In previous studies of *non-acidotic diabetics* (24) no difference was found in SILA values between juvenile and older non-adipose patients, whereas the adipose diabetics had higher SILA values than the others. Clinically the juvenile and the older non-adipose diabetics do not resemble each other the present study was started, therefore in the hope that the two groups of diabetics would show a distinction with respect to the occurrence of insulin antagonism in serum. The result was that with the rat epididymal fat method no insulin antagonism could be demonstrated in any of the groups of diabetics mentioned. A study of the insulin recovery from serum from poorly regulated juvenile diabetics without ketonuria also showed no insulin antagonism. This is of interest, as Vallance-Owen et al. (31) have shown that when examined by the rat diafragm method serum from such patients contains an insulin antagonist. Vallance Owen has subsequently outlined a technic for extracting the antagonist, which is associated with the serum albumin (32) Using the rat diafragm method, it has been possible with the above technic to show insulin antagonism in the albumin fraction in other types of diabetics and

Table VIII Insulin addition to serum from patients with acromegaly

Assay no.	Patient no.	Serum glucose mg	SILA Undiluted serum		SILA Diluted serum		No. of rats	Index of precision
			μU/ml	+ 100 μU/ml	μU/ml	+ 100 μU/ml		
289	189	109	180	480	39	370	4	0.29
480	231	82	220	360	35	470	4	0.13
891	271	87	200	370	68	235	4	0.23
714	270	202	145	282	64	175	4	0.27

These values have not been multiplied by the dilution factor

obvious inhibition of the added insulin could be demonstrated also on these occasions, even with the addition of 1 000 μU insulin per ml diluted serum (table V)

Serum from examination III was subjected to electrophoresis in a polyvinyl chloride block. Demonstrable insulin-like activity was seen in all the protein fractions examined with the exception of the albumin and the slowest part of the γ-globulin fraction The insulin like activity corresponding to the β-globulins was the greatest The added insulin was demonstrably inhibited in three of the serum protein fractions examined corresponding to the β-globulins and the most rapid γ-globulins. This inhibition was present even when these fractions were dissolved in a buffer volume 6—8 times greater than the serum volume which underwent electrophoresis (table VI)

Experiments were carried out on the addition of insulin to sera from 5 patients with *diabetic acidosis* (table VII) one of them with diabetic coma (no 206) and one with precoma (no. 183) Four of these five patients had insulin like activity in the serum an average value of 95.2 μU/ml being found in undiluted serum and 310 μU/ml in diluted serum.

In undiluted serum a mean increase in SILA of 32 μU/ml was found after adding 100 μU/ml. This rise is significantly less than 100 μU/ml ($p < 0.05$) In diluted serum the mean increase in SILA was 290 6 μU/ml which is significantly greater than 100 μU/ml ($p < 0.05$) An examination of acidotic diabetics, using the rat epididymal fat method thus shows insulin antagonism in undiluted serum which cannot be demonstrated in diluted serum On the contrary the rise in SILA in diluted serum was three times greater than the amount of insulin added.

The opportunity arose to examine four patients with *acromegaly* one of them with diabetes mellitus. None of the sera showed any inhibition of the added insulin (table VIII)

Discussion

In the present investigation, an attempt was made to demonstrate insulin antagonism in a number of normal subjects and diabetics, by adding small amounts of insulin to serum samples. It proved possible to demonstrate such an antagonism in serum from patients with diabetic acidosis and in one patient with insulin resistance.

In a previous study using the rat epididymal fat method, it was found that after adding insulin to normal serum the rise in SILA was greater than corresponded to the amount of insulin added, 100 μU/ml (21)

Other investigators report that using the rat epididymal fat method, the added insulin amount is recovered quantitatively (13, 17 25) In the present study the experiments were carried out with a technic which deviated from that employed in the above-mentioned previous study In the earlier experiments when the insulin was added to the serum the pipette was emptied by blowing out. This blowing out which caused a weak formation of foam in the serum, was omitted in the present experiments. Using this new technic, it was shown, that when pig insulin is added to *undiluted serum* from *normal subjects* and *normal pigs* the SILA increases correspond to the amount of insulin added. This difference between the first and subsequent experiments may reasonably be explained as the result of altering the technic used in adding the insulin.

In the present study when insulin was added to *diluted serum from pigs* a rise in SILA was found which was greater than the amount of insulin added. Likewise, in the experiments with *diluted human serum*, the average value of the insulin recovered was also greater than expected although the rise was not statistically significant. Staub (28) also using the rat epididymal fat method, has likewise found an increased insulin recovery on adding insulin to diluted serum.

The cause of the increased recovery of insulin from diluted serum is unknown. Some studies seem to show however that the SILA value in undiluted serum measured by the rat diaphragm method and by the rat epididymal fat method constitutes only a part of the insulin amount present in the serum. Part of the serum insulin appears to be bound to protein in an inactive form (1 2) It is conceivable that by adding insulin to diluted serum in which the normal equilibrium between free and bound insulin is presumably disturbed thus equilibrium is so displaced that endogenous insulin is released The addition of insulin would result in the SILA value being increased by more than the amount of insulin added.

In previous studies of *non-acidotic diabetes* (24) no difference was found in SILA values between juvenile and older non-adipose patients, whereas the adipose diabetics had higher SILA values than the others. Clinically the juvenile and the older non adipose diabetics do not resemble each other the present study was started therefore in the hope that the two groups of diabetics would show a distinction with respect to the occurrence of insulin antagonism in serum. The result was that with the rat epididymal fat method, no insulin antagonism could be demonstrated in any of the groups of diabetics mentioned. A study of the insulin recovery from serum from poorly regulated juvenile diabetics without ketonuria also showed no insulin antagonism This is of interest, as Vallance-Owen et al. (31) have shown that, when examined by the rat diaphragm method serum from such patients contains an insulin antagonist. Vallance Owen has subsequently outlined a technic for extracting the antagonist, which is associated with the serum albumin (32) Using the rat diaphragm method, it has been possible with the above technic to show insulin antagonism in the albumin fraction in other types of diabetics, and

even in normal subjects, although in a lesser amount (34). After extraction by Vallance-Owen's method and using the rat epididymal fat method it has not been possible in the present study to show insulin antagonism in the albumin from poorly regulated juvenile diabetics. Vallance Owen's antagonist thus seems to be inactive on rat epididymal fat both in the case of untreated serum and extracted albumin. This finding is in agreement with a recently published study (20) in which it is shown that albumin when prepared from normal serum as indicated by Vallance Owen, inhibits the insulin effect on the glucose uptake by the rat diafragm but does not inhibit the insulin effect on the oxidation of glucose-1 C^{14} by rat epididymal fat.

The fact that Vallance Owen's antagonist is active with rat diafragm but not with rat epididymal fat is of considerable interest. It suggests that this antagonist does not act by binding insulin in serum in the same way as the insulin antibody demonstrated in serum from insulin requiring patients and guinea pigs (3 26).

In previous studies, insulin antagonism in serum from patients in diabetic acidosis has been demonstrated by recording the action of insulin on both glucose uptake by and glycogen deposition in the rat diafragm (8 10 14 36). Electrophoretic separation of the serum proteins showed that the antagonist was localized to α_1 α_2-globulin (9). It has been discussed whether the antagonist appearing in diabetic acidosis is the same as that demonstrated by Vallance-Owen in the albumin fraction from poorly regulated non-ketotic juvenile diabetics (33).

In the present investigation, insulin antagonism in serum from acidotic diabetics has been demonstrated by means of the rat epididymal fat method. However the Vallance-Owen antagonist did not inhibit the action of insulin on rat epididymal fat, when the insulin was added either to untreated serum or to serum after the extraction of the antagonist. This investigation suggests, therefore, that the antagonist occurring during diabetic acidosis is not the same as that described by Vallance-Owen. The presence of the antagonist is probably the reason why the SILA values in undiluted serum from the acidotic diabetics were normal in contrast with the elevated values in diluted serum in which no antagonism was found. It probably also explains the observation of Steinke et al. (29) who found that SILA in diluted serum from acidotic diabetics was low when it was measured by the rat diafragm technic, but high when the fat pad was used.

In view of the significance attached to the growth hormone in the pathogenesis of diabetes, it is interesting that in the present study no insulin antagonism could be demonstrated in the serum from four patients with active acromegaly one of whom had diabetes mellitus. Field and Rigby (11) have shown that in the serum of 3 out of 4 patients with diabetes and acromegaly insulin antagonism could be demonstrated by the rat diafragm method. In this study they used the same rat-diafragm technic as in the studies in which insulin antagonism could be demonstrated in serum from diabetics in acidosis. Kipnis et al. (19) using the rat epididymal fat method found no insulin antagonism in the serum from two patients with diabetes and acromegaly. These results are however not incompatible with the assumption that in some diabetics with acromegaly an insulin antagonist can be found in serum, active on both rat epididymal

fat and rat diafragm. It must be assumed that there are two types of diabetes among acromegalics, one due to the acromegaly and the other the result of a chance coincidence of acromegaly and diabetes mellitus. It is reasonable to suppose that these two types of diabetes differ with respect to insulin antagonism in serum. Further studies of insulin antagonism in patients with diabetes and acromegaly elucidated by means of both the rat diafragm method and the epididymal fat method, would therefore be of consider able interest.

Previous studies with I^{131}-labelled insulin have shown that in man, insulin antibodies appear after 3—4 months of insulin treatment (3 33) With the technic used here, in which a small amount of insulin is added to serum, it has been possible to demonstrate insulin antag onism in only one out of six insulin-treated diabetics. This one patient de veloped insulin resistance, nd the insulin antagonism could be demonstrated in both undiluted and diluted serum before, during, and after insulin resistance had become manifest, that is to say also at a stage when the daily dose of insulin was less than 200 I U per 24 hours.

In the present study therefore, using the rat epididymal fat method, t has been possible to demonstrate insulin antagonism only in serum from diabetics with acidosis or with insulin resistance. In spite of the insulin antagonism revealed there was demonstrable SILA in undiluted serum from 5 of these 6 patients. This state of affairs remains unexplained. It might be considered to support the view that, besides insulin, serum contains other factors which are able to increase the oxidation of glucose-1-C' by rat epididymal fat. The investigation of the serum protein fractions from the patient with insulin resistance shows, how ever that the β-γ-globulins, which contain the insulin antagonist, contain at the same time the maximum insulin-like activity As a series of investigations show in fact that the β-γ-globulins in insulin-treated diabetics contain the antibody which binds insulin (4 7 12) this find ing supports the assumption that the insulin like activity found in these frac tions is due to insulin. When both insulin antagonism and insulin-like activity can be demonstrated in the β-γ-globulins this presumably must be because the insulin antagonist is unable to inhibit the effect of all biologically active insulin.

It is remarkable that in undiluted serum from a series of diabetic subjects the rise in SILA found after the addition of insulin has been greater than the activity corresponding to the amount of insulin added. With the technic employed, this phenomenon has not been observed in normal subjects. So far no explanation can be given for these observations. It is possible, however that investigations which have been started on the occur rence of insulin-like activity in serum protein fractions from normal subjects and diabetics, will throw light on this problem.

Summary

In the present study the rat epididymal fat method has been used to investigate the insulin recovery in the serum from normal persons and diabetics.

With this technic insulin antagonism was found in the serum from acidotic diabetics. In diluted serum (20 %) from these patients no insulin antagonism was found.

No insulin antagonism was found in the serum from normal persons or from

even in normal subjects although in a lesser amount (34) After extraction by Vallance Owen's method and using the rat epididymal fat method it has not been possible in the present study to show insulin antagonism in the albumin from poorly regulated juvenile diabetics. Vallance-Owen's antagonist thus seems to be inactive on rat epididymal fat, both in the case of untreated serum and extracted albumin. This finding is in agreement with a recently published study (20) in which it is shown that albumin, when prepared from normal serum as indicated by Vallance-Owen, inhibits the insulin effect on the glucose uptake by the rat diafragm but does not inhibit the insulin effect on the oxidation of glucose-1 C^{14} by rat epididymal fat.

The fact that Vallance Owen's antagonist is active with rat diafragm but not with rat epididymal fat is of considerable interest. It suggests that this antagonist does not act by binding insulin in serum in the same way as the insulin antibody demonstrated in serum from insulin requiring patients and guinea pigs (3 26)

In previous studies, insulin antagonism in serum from patients in diabetic acidosis has been demonstrated by recording the action of insulin on both glucose uptake by and glycogen deposition in the rat diafragm (8, 10 14 36) Electrophoretic separation of the serum proteins showed that the antagonist was localized to α α-globulin (9) It has been discussed whether the antagonist appearing in diabetic acidosis is the same as that demonstrated by Vallance-Owen in the albumin fraction from poorly regulated non ketotic juvenile diabetics (33)

In the present investigation, insulin antagonism in serum from acidotic diabetics has been demonstrated by means of the rat epididymal fat method. However the Vallance-Owen antagonist did not inhibit the action of insulin on rat epididymal fat, when the insulin was added either to untreated serum or to serum after the extraction of the antagonist. This investigation suggests, therefore, that the antagonist occurring during diabetic acidosis is not the same as that described by Vallance-Owen. The presence of the antagonist is probably the reason why the SILA values in undiluted serum from the acidotic diabetics were normal in contrast with the elevated values in diluted serum, in which no antagonism was found. It probably also explains the observation of Steinke et al (29) who found that SILA in diluted serum from acidotic diabetics was low when it was measured by the rat diafragm technic, but high when the fat pad was used.

In view of the significance attached to the growth hormone in the pathogenesis of diabetes, it is interesting that in the present study no insulin antagonism could be demonstrated in the serum from four patients with active acromegaly one of whom had diabetes mellitus. Field and Rigby (11) have shown that in the serum of 3 out of 4 patients with diabetes and acromegaly insulin antagonism could be demonstrated by the rat diafragm method. In this study they used the same rat-diafragm technic as in the studies in which insulin antagonism could be demonstrated in serum from diabetics in acidosis Kipnis et al. (19) using the rat epididymal fat method, found no insulin antagonism in the serum from two patients with diabetes and acromegaly These results are however not incompatible with the assumption that in some diabetics with acromegaly an insulin antagonist can be found in serum, active on both rat epididymal

fat and rat diafragm. It must be assumed that there are two types of diabetes among acromegalics, one due to the acromegaly and the other the result of a chance coincidence of acromegaly and diabetes mellitus. It is reasonable to suppose that these two types of diabetes differ with respect to insulin antagonism in serum. Further studies of insulin antagonism in patients with diabetes and acromegaly elucidated by means of both the rat diafragm method and the epididymal fat method, would therefore be of considerable interest.

Previous studies with I^{131}-labelled insulin have shown that in man insulin antibodies appear after 3—4 months of insulin treatment (5, 35). With the technic used here, in which a small amount of insulin is added to serum, it has been possible to demonstrate insulin antagonism in only one out of six insulin-treated diabetics. This one patient developed insulin resistance, and the insulin antagonism could be demonstrated in both undiluted and diluted serum, before, during, and after insulin resistance had become manifest, that is to say also at a stage when the daily dose of insulin was less than 200 I.U. per 24 hours.

In the present study therefore, using the rat epididymal fat method, it has been possible to demonstrate insulin antagonism only in serum from diabetics with acidosis or with insulin resistance. In spite of the insulin antagonism revealed, there was demonstrable SILA in undiluted serum from 5 of these 6 patients. This state of affairs remains unexplained. It might be considered to support the view that, besides insulin, serum contains other factors which are able to increase the oxidation of glucose-1-C^{14} by rat epididymal fat. The investigation of the serum protein fractions from the patient with insulin resistance shows, however, that the β-γ-globulins which contain the insulin antagonist, contain at the same time the maximum insulin-like activity. As a series of investigations show in fact that the β-γ-globulins in insulin-treated diabetics contain the antibody which binds insulin (4, 7, 12) this finding supports the assumption that the insulin-like activity found in these fractions is due to insulin. When both insulin antagonism and insulin-like activity can be demonstrated in the β-γ-globulins this presumably must be because the insulin antagonist is unable to inhibit the effect of all biologically active insulin.

It is remarkable that in undiluted serum from a series of diabetic subjects the rise in SILA found after the addition of insulin has been greater than the activity corresponding to the amount of insulin added. With the technic employed this phenomenon has not been observed in normal subjects. So far no explanation can be given for these observations. It is possible, however, that investigations which have been started on the occurrence of insulin-like activity in serum protein fractions from normal subjects and diabetics, will throw light on this problem.

Summary

In the present study the rat epididymal fat method has been used to investigate the insulin recovery in the serum from normal persons and diabetics.

With this technic insulin antagonism was found in the serum from acidotic diabetics. In diluted serum (20 %) from these patients no insulin antagonism was found.

No insulin antagonism was found in the serum from normal persons or from

newly diagnosed diabetics without acidosis.

In the serum from normal persons and from normal pigs with added insulin the recovery was about 100 %. When insulin was added to serum from old diabetics the recovery was more than 100 %.

The insulin antagonist described by Vallance-Owen was isolated from juvenile diabetics without acidosis. It did not inhibit the insulin effect on the rat epididymal fat.

One patient with insulin resistance had insulin antagonism in the serum. The maximum insulin like activity was found in the β-γ-globulins where the insulin antagonist was also located.

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Observations on the Effect of Varying Ventricular Rate on the Circulation at Rest and during Exercise in two Patients with an Artificial Pacemaker

By

S. Bevegård

A number of investigators have studied the circulation in patients with complete heart block (8, 18, 19 20, 21 23 25) Patients who have repeated attacks of Adams-Stokes syndrome or are disabled because of slow ventricular rates are now often treated with artificial internal pace making (7 9, 10 22, 29)

According to some investigations on dogs an artificial increase of the heart rate by atrial (2, 26) or ventricular (28) stimulation causes a decrease of the stroke volume. When the heart rate in man has been increased by administration of an anticholinergic substance the stroke volume has been found to decrease (3, 4 11) or remain unchanged (30)

Patients with an artificial pacemaker offer the opportunity to study the effect on the circulation of varying heart rates at rest and during exercise. Since some types of implantable pacemakers have fixed stimulating frequency it is important to find out the heart rate which is most suitable for both resting and exercise conditions.

Case reports

Some anthropometric data for the two cases are presented in table I

Case 1 57-year-old man. Diphtheria without known complications at the age of 13. For the past year repeated attacks of fainting. ECG on different occasions showed atrioventricular block of varying degree. For the last months constant complete atrioventricular block with ventricular rate varying between 25 and 33 beats/min. No history of congestive heart failure. The etiology is obscure Artificial pacemaking was indicated because of repeated Adams-Stokes seizures. Electrodes were sutured into the myocardium and attached to an artificial pacemaker

Case 2. 41-year-old, previously healthy man who suddenly developed a complete atrioventricular block with a ventricular rate varying between 15 and 50 beats/min. The etiology is uncertain, but perimyocarditis

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Table I Some anthropometric data for 2 patients with complete heart block treated with artificial pacemaker

Case no.	Age years	Height cm	Weight kg	B. S. A. m	Heart volume ml	Total hemoglobin g	Blood volume l
1	57	172	75	1.88	940	827	6.2
2	41	172	57	1.69	1 040	697	5.0

was suspected Repeated attacks of Adams-Stokes syndrome were considered to be an indication for artificial pacemaking and electrodes were sutured to the myocardium and connected to an external pacemaker

Methods and procedure

The methods employed for determination of heart volume total hemoglobin and blood volume have been the same as previously reported (5)

Right heart catheterization was performed by a percutaneous technic from the femoral vein in case 1 and from an exposed cubital vein in case 2 With the percutaneous technic a teflon catheter is introduced according to Seldinger (27) During screening the tip is advanced to the right atrium just above the tricuspid orifice. A thin polyethylene catheter (size PE 50) is then passed through the teflon catheter and is allowed to follow the blood stream into the right ventricle and pulmonary artery Through the thin catheter blood samples can be drawn slowly and undamped pressures can be recorded. With the Seldinger technic the brachial artery was also cannulated Cardiac output was determined according to the direct Fick method with the same technic as previously described from this laboratory (5) The ventricular rate was changed stepwise with the pacemaker Determinations of pressures and cardiac output were made 10 min after changing the heart rate at rest and after 5 min during exercise The patients were studied in the supine position both at rest and during exercise The exercise was performed on a bicycle ergometer at a load of 300 kpm/min. The reference level for zero pressure was 5 cm below the insertion of the fourth rib at the sternum. No complications occurred. In case 1 pressures and cardiac output were determined at heart rates of 22, 31 52, 74 and 109 beats/min. at rest and at heart rates of 75 and 109 beats/min during work. In case 2, determinations were performed at heart rates of 28, 55, 72 91 and 95 beats/min. at rest and 56 and 100 beats/min. during work.

Results

The *total amount of hemoglobin* and the *blood volume* were normal in relation to body weight in both cases (15 16) The *heart volume* in the prone position was normal in relation to the total amount of hemoglobin in case 1 but larger than normal in case 2.

The *data obtained in connection with right heart catheterization* are presented in table II and fig 1

The *oxygen uptake* at rest varied between + 1 % and + 14 % of the predicted basal value (13) in case 1 and between — 1 % and + 12 % in case 2 The oxygen uptake during work at 300 kpm/min increased to 951 ml/min in case 1 and to 798 ml/min. in case 2, corresponding to mechanical efficiencies of 20 and 24 % respectively

The *oxygen saturation of the arterial blood* was within normal limits at rest and during work in both cases.

The *cardiac output* at rest was within normal limits in relation to oxygen uptake (5 12 17) at heart rates within the range of 70 to 110 beats/min. At the lower heart rates studied, the cardiac output decreased to abnormally low values in spite of increasing stroke volume (fig

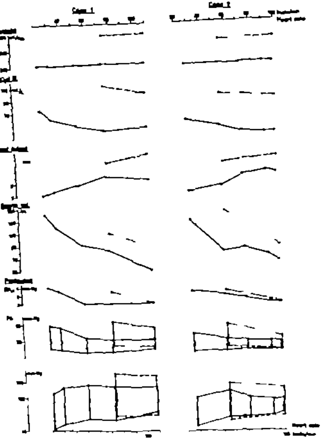


Fig. 1 The effect of artifical variations in heart rate on some circulatory functions at rest (filled circles connected by full lines) and during exercise (open circles connected by broken lines).

RAm = right atrium (mean)
PA = pulmonary artery
Br. A = brachial artery

1) During exercise the cardiac output was at the lower limits of the normal variation in relation to oxygen uptake. At the same heart rate it was higher during exercise than at rest due to a corresponding increase of the stroke volume. Increase of the heart rate during exercise caused only a slight increase of the cardiac output (fig. 1)

The *arterio-venous oxygen difference* varied inversely with the cardiac output because the oxygen consumption showed fairly constant levels with different heart rates at rest and at the two heart rates during exercise (fig 1)

The *stroke volume* at rest was largest at the lowest heart rates. At heart rates within the range of 50—70 beats/min. it showed less marked changes, but otherwise it appeared to decrease continuously with increasing heart rate (fig 1) The stroke volume during exercise was markedly larger than at rest at the same heart rate. Also during exercise the stroke volume decreased when the heart rate was increased. The stroke volume at rest was within normal ranges in relation to heart volume (5 17) only at the lowest heart rates.

Intracardiac and intravascular pressures (fig 1 table II) The pressures in the brachial artery systolic, diastolic and mean, rose when the heart rate was increased up to around 50 beats/min. With a further rise

Table II The effect of varying heart rates on the circulation in supine position at rest and during exercise in

Case no.	Work load kpm/min.	Heart rate beats/min.	Oxygen uptake ml/min.	O_2-capacity ml/100 ml	O_2-sat. %		AV-O_2-diff. ml/l
					Br.A	PA	
1	Rest	31	223	19 1	97	67	61
	Rest	22	233	18.8	99	60	75
	Rest	52	235	18.6	97	72	49
	Rest	74	251	18.6	97	76	41
	Rest	109	251	19.2	98	75	46
	300	75	931	19.9	97	43	112
	300	109	951	20.2	96	48	99
2	Rest	55	201	19.1	99	77	45
	Rest	28	204	18.9	99	71	56
	Rest	72	215	19.3	98	80	36
	Rest	91	228	19 4	98	82	34
	Rest	99	218	19.3	99	82	35
	300	56	775	20.2	99	53	96
	300	100	798	20.6	99	56	90

Br.A = brachial artery PA = pulmonary artery RA = right atrium.

in heart rate the mean pressure increased slightly but the pulse amplitude decreased The brachial arterial pressures in relation to age were normal both at rest and during exercise (5 12) The systolic pressure in the pulmonary artery was highest at the lowest heart rates and decreased with increasing heart rate — markedly at first and then very slightly In case 1 the diastolic and the mean pressures decreased when heart rate was increased to 52 beats/min. and then rose slightly with further increase of heart rate. In case 2 the diastolic and the mean pressures in the pulmonary artery showed only minor changes but tended to increase at the highest heart rate. Also during exercise the pressure in the pulmonary artery decreased when the heart rate was increased The pulse amplitude tended to decrease in parallel with the stroke volume The mean pressure in the pulmonary artery appeared to change in the same direction as the mean pressure in the right atrium. The pressure in the pulmonary artery was within normal ranges with regard to age both at rest and during exercise. The mean pressure in the right atrium was normal. It appeared to decrease with increasing heart rate. In case 1 at rest it was about zero at heart rates between 52 and 109 beats/min

Discussion

The two patients studied were treated with artificial pacemakers because of complete atrioventricular block with repeated Adams-Stokes seizures. The etiology is uncertain and it cannot be determined whether the underlying cardiac disease has caused myocardial changes which could modify the circulatory response to artificial variation in heart rate. The incoordination between atrial and

[illegible] patients with an artificial pacemaker

Card. output l/min.	Stroke volume ml	Pressures, mm Hg							Peripheral vascular resistance index
		RA	PA			Br.A.			
		M	S	D	M	S	D	M	
3.7	120	3	35	8	16	144	54	76	38
5.1	148	4	38	10	17	130	44	64	39
4.8	92	0	23	5	11	151	68	94	37
6.1	83	0	21	6	12	148	76	101	31
5.5	50	0	20	9	14	146	85	111	38
8.3	111	3	43	14	30	179	76	115	26
9.6	88	0	35	12	22	172	77	108	21
4.4	80	2	21	7	13	137	73	91	33
3.6	120	5	28	6	13	119	51	70	33
6.0	84	1	19	8	12	120	74	93	26
6.7	73	0	18	7	11	115	77	94	24
6.3	64	0	18	8	13	117	85	96	26
8.1	144	3	39	12	22	154	65	91	19
8.5	89	0	24	8	15	145	77	96	18

M [illegible] mean. S = systolic. D = diastolic.

ventricular contractions and the abnormal mode of ventricular activation by means of stimulating electrodes are also factors which limit the transference of the present results to normal conditions. The abnormal mode of ventricular activation did *not* cause any demonstrable deviations from the normal duration of mechanical systole as measured from the phonocardiogram (4).

None of the patients had any history of congestive heart failure. The size of the heart was normal in relation to the total amount of hemoglobin (15) in case 1 but large in case 2. In neither case however was the heart larger than could be explained by adaptation to a large stroke volume during the periods of slow ventricular rate. It also appeared that at lower ventricular rates the stroke volume showed a normal relationship both to heart volume and to blood volume (5, 17). Adaptation of the circulation to heart block with slow ventricular rate is accomplished by an increase of heart volume and stroke volume, whereby a fairly normal cardiac output can be maintained (18).

In the two cases studied in this report, the stroke volume at rest was largest at the lowest ventricular rate. With increasing ventricular rate both the mean pressure in the right atrium and the stroke volume decreased. The mean pressure in the pulmonary artery showed the same general tendency to decrease as did the right atrial pressure, which might be a sign that left ventricular filling pressure changed in the same direction as right ventricular filling pressure. The findings could be explained by a redistribution of blood from the central to the peripheral circulation with increasing ventricular rate. The lack of coordination between atrial and ventricular contractions should also tend to decrease the stroke volume

Table II The effect of varying heart rates on the circulation in supine position at rest and during exercise in

Case no.	Work load kpm/min.	Heart rate beats/min.	Oxygen uptake ml/min.	O_2-capacity ml/100 ml	O_2 sat. %		AV-O_2-diff. ml/l
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Discussion

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made on dogs by Warner and Toronto (28). The mechanisms already discussed in connection with the observations at rest could also satisfy the observations during exercise.

Summary

Heart catheterization was performed in two patients with an artificial pacemaker. The circulatory effects of artificial variations in heart rate were studied at rest and during exercise. The stroke volume was largest at the lowest ventricular rate and appeared to decrease with increasing ventricular rate. Within the range of 50 to 70 ventricular beats/min. the changes in stroke volume were less marked. The stroke volume was higher during exercise than at rest at the same ventricular rates. The regulatory mechanisms are discussed. The mean pressure in the right atrium appeared to change in the same direction as the stroke volume both at rest and during exercise. This might indicate that in these cases change in ventricular rate per se could influence the distribution of the blood volume and thus also ventricular filling and stroke volume. The increase in stroke vol me and right atrial mean pressure with leg exercise might be a consequence of redistribution of blood in a central direction.

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with increasing ventricular rate, since atrial systole becomes more important for ventricular filling when diastole shortens. However this lack of coordination can not be expected to decrease the mean pressure in the right atrium. It also seems unlikely that this is the only explanation for the considerable changes in stroke volume occurring at low ventricular rates. It is more plausible to explain the decrease of ventricular filling pressure and stroke volume, with increasing ven tricular rate by a redistribution of the blood volume. A regulatory response elicited via the pressor receptors could also be part of the underlying mechanism Carlsten et al. (6) found that stimulation of the carotid sinus nerve in man caused slowing of heart rate, peripheral vasodilatation and also reduced the pressure pulse amplitude, which they ascribe to a decrease of the stroke volume. A reduc tion in sympathetic outflow might influence the volume of venous capacitance vessels and cause a shift of blood to the periphery (14) and thus decrease ven tricular filling and stroke volume In these two cases the increase of the mean blood pressure at rest when ventricular rate was augmented presents an enhanced stimulus to the pressor receptors. Con comitant variation in sympathetic tone should be reflected in changes of atrial rate. When the ventricular rate was augmented in case 1 from 22 to 74 beats/min the atrial rate decreased from 85 to 67 beats/min. In case 2 atrial rate de creased from 76 to 49 beats/min when ventricular rate increased from 28 to 90 beats/min. That atrial rate varies in versely with ventricular rate at rest has also been observed in other cases with complete heart block. These variations in atrial rate probably represent changes in sympathetic tone. The observations in this report are in agreement with those made by Abrams et al. (1) and Müller and Bellet (24) who studied the circulation in one patient each during artificial pacemaking at rest and found a decrease of the stroke volume with increasing heart rate. Warner and Toronto (28) made the same observation on closed-chest dogs, when atrioventricular conduction had been interrupted They suggest that "powerful control mechanisms exist which are capable of bringing about adjustments in stroke volume in response to a change in heart rate such that cardiac output will be held constant over a wide range of heart rates In these two cases cardiac output was virtually unchanged when ventricular rate varied between 72 and 109 beats/min.

During exercise the stroke volumes were significantly larger than at rest at the same ventricular rate. The mean pressure in the right atrium followed approximately the changes in stroke volume (fig 1) An increase of the ventricular rate during exercise caused a decrease of stroke volume and mean pressure in the right atrium and pulmonary artery The increase in right atrial mean pressure on transition from rest to exercise with constant ventricular rate indicates im proved ventricular filling which could be a result of redistribution of blood to the central circulation, accomplished by the venous pump of the leg muscles. Regulatory mechanisms such as increased sympathetic outflow may also take part in this adaptation. As was the case at rest an increase in ventricular rate during exercise caused a decrease of the mean pressures in the right atrium and pulmonary artery and of the stroke volume such that cardiac output increased only slightly The changes during exercise are also in agreement with the experiments

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Circulatory Response to Recumbent Exercise and Head up Tilting in Patients with Disturbed Sympathetic Cardiovascular Control (Postural Hypotension)

Observations on the Effect of Norepinephrine Infusion and Antigravity Suit Inflation in the Head-up Tilted Position

By

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Adequate blood supply to the tissues is normally secured through reflex cardiovascular adjustments. Postural hypotension, described by Bradbury and Eggleston in 1925 (4) is a rare disturbance of the reflex cardiovascular regulation of arterial blood pressure. The underlying lesion is believed to be organic and to have central localization (6, 15, 24, 30, 32). The most apparent and easily demonstrated manifestation of postural hypotension is a marked fall in arterial blood pressure with no increase in heart rate on head-up tilting or at best only a slight increase. A low urinary excretion of norepinephrine has been found in patients with this disease (1, 22). Hickler et al. (19, 20) demonstrated a failure in the normal increase of plasma norepinephrine levels on head-up positioning. Postural hypotension is a condition eminently suited for analysis of the significance for different circulatory demands of cardiovascular control by the central reflex sympathetic mechanism. Only in a few instances have the hemodynamics of these cases been studied with the aid of cardiac catheterization (18, 28, 29) or with dye dilution technique (3). Altogether these reports include only 6 patients with idiopathic postural hypotension.

In our present report the circulation was studied in four patients with idiopathic postural hypotension. Our purpose was to analyse the influence of exercise and body position on the circulatory dynamics. We have included observations on attempts to normalize the circulatory response to upright tilting. This was accomplished by administering norepinephrine, and also by counteracting the

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Addendum

Since completion of this article three additional cases have been studied. They showed the same circulatory response to changes in ventricular rate as the two cases reported in this paper

showed right bundle branch block. Occasional attacks of atrial fibrillation since 1935. In the sitting position he could perform exercise at a load of 400 kpm/min. for 4 min., but was then near fainting. In the supine position he could perform 400 kpm/min. but became extremely fatigued. The pulse rate increased by 36 beats/min. from rest to exercise.

Case 4 60-year-old man with typical postural hypotension for several years. No signs of diabetes. The ECG was normal at rest and during work. H could not perform any exercise in the sitting position. In the supine position he could work 6 min. at 130 kpm/min. and was then extremely tired. The pulse rate increased by only 5 beats/min. from rest to exercise.

Methods

The methods for determination of total amount of hemoglobin, blood volume, and heart volume in the prone position and for the exercise test have been the same as earlier reported (2)

Right heart catheterization was performed according to the technique used in this laboratory (2) A double-lumen catheter was employed. During the whole procedure zero pressure was measured at the mid-thoracic point of an antero-posterior plane through the 4th intercostal space. Cardiac output was determined with the direct Fick method. Cases 2—4 were investigated a second time for the purpose of studying the circulatory adaption to head-up position after infusion of norepinephrine in varying doses and after application of the inflated antigravity suit. During the second investigation the dye dilution technique was also used for determination of cardiac output and central blood volume. The indicator dye, cardiogreen, was injected rapidly with calibrated syringe into the main stem of the pulmonary artery and sampling took place at the root of the ascending aorta via polyethylene catheter inserted by percutaneous technique (26 from the brachial artery The blood from the sampling set passed through a cuvette densitometer (XC-100A, Waters Co.) at speed kept constant t about 20 ml/min. by the aid of motor-driven syringe. The concentration-time curves and signal for the dye injection were recorded on Speedomax recorder The cardiac output and the central blood volume were calculated according to the Stewart Hamilton equations (14 31) The infusion of norepinephrine was made into the right ventricle through the end opening of the double-lumen catheter The infusion was kept constant with great accuracy by means of a motor-driven injection syringe. For recording of right ventricular pressure the infusion was interrupted during short periods.

In order to study the effect of blood volume redistribution, while the patient was tilted head-up, an antigravity suit of the type used in the Swedish air force was applied and inflated to pressure of 40 mm Hg

Procedure during heart catheterization

The patients received no premedication. All drugs used for treatment of their disease had earlier been withdrawn for sufficient time so as not to influence the results of the study When the catheters had been introduced pressures and cardiac output were measured at rest and during work (cases 2—4) in the reclining position. Thereafter the subjects rested for about 30 min and were then placed on a tilt table. In cases 3 and 4 measurements were again performed at rest in the supine position. The patients were then studied in a head-up position tilted at an angle of 30—45° before and during constant infusion of norepinephrine. Cases 2—4 were reexamined some time later The effect of antigravity suit inflation and of norepinephrine infusion (I two different doses) was then determined in the up-tilted position.

Results

The ability to perform muscular exercise was also low for all cases while in the supine position. All patients had to stop the exercise already at low heart rates because of severe general fatigue The highest heart rate achieved during recumbent exercise was 109 beats/min. *Total amount of hemoglobin* (THb) per kg body weight was large in case 1 It was somewhat low in case nd also in case 4 on the second investigation as compared with the values

Table I. Some anthropometric data for 4 male subjects with postural hypotension

Case no.	Age years	Height cm	Weight kg	B.S.A. m²	Heart volume ml	Total hemoglobin g	Hemoglobin conc. g/100 ml	Blood volume l	Pulse rate at rest beats/min. (supine)	Highest pulse rate during work beats/min. (supine)	Work load performed in supine position
1	61	176	56.4	1.69	640	1 016	12.1	8.4	70	79	300
2 a	64	182	79.2	2.02	1,030	735	13.0	5.7	54	109	600
2 b	65	182	80.0	2.03	1 040	772	11.6	6.7	—	—	—
3 a	67	173	62.9	1.76	1,040	712	12.1	5.9	64	100	400
3 b	68	173	63.0	1.76	—	—	—	—	—	—	—
4 a	60	175	57.9	1.72	970	669	9.5	6.9	69	74	150
4 b	61	174	61.0	1.76	930	503	9.5	5.3	—	—	—

a = values at the first investigation, b = at the second.

blood volume displacement by inflating an antigravity suit worn by the patient. Reference is also made herein to the significance of the disturbed sympathetic regulation for the behavior of the stroke volume.

Material

Four male subjects with postural hypotension were studied. Values for age, body weight, height and other anthropometric data are presented in table I. Further studies in these cases have been performed at the Department of Endocrinology, Karolinska sjukhuset (head R. Luft) and the peripheral circulation has been analyzed at the Department of Clinical Physiology, Akademiska sjukhuset, Uppsala (head G. Strom). These results will be reported separately. All patients presented the typical picture of postural hypotension with *prompt* fall of arterial pressure and with no increase in heart rate on head-up tilting or only a slight increase.

Case reports

Case 1. 64-year-old man. Since 1954 occasional attacks of fainting coming on while in the erect position. Mild diabetes with traces or small amounts of glucose in the urine but normal fasting glucose level in blood. No signs of any disease involving the central nervous system. The ECG was normal. At an attempt to exercise while sitting on a bicycle ergometer he fainted 1 1/2 min. after exercising with a load of 100 kpm/min. In the supine position he could perform a 6 min. exercise at 300 kpm/min. The pulse rate had increased by only 9 beats/min. between rest and the end of the exercise period.

Case 2. 64-year-old man with typical symptoms of postural hypotension. There were no signs of diabetes or any disease involving the central nervous system. Had attacks of fainting while in the erect position. After walking 10—20 m he was fatigued with dim vision and resorted to squatting. In the supine position he could perform work amounting to 600 kpm/min. The pulse rate increased by 55 beats/min from rest to exercise. The ECG was normal at rest but showed slight depression of ST-segments during and after supine exercise.

Case 3. 67 year-old man. In 1956 had repeated attacks of fainting. During one attack he sustained a head injury with a skull fracture. He has, however, never shown any signs of central nervous system involvement. Since 1956 he has presented a typical picture of postural hypotension. No diabetes. The ECG

the first ( ) and the second (b) investigation

roke i- me l	Pressures, mm Hg									Peripheral vascular resistance index	Central blood volume l
	Right ventricle		Pulmonary artery			Pulmonary capillary venous	Brachial artery				
	Systolic	End-diastolic	Systolic	Diastolic	Mean		Systolic	Diastolic	Mean		
98	21	2	20	4	9	7	117	67	92	22	—
54	10	−5	10	1	5	0	85	53	64	23	—
100	37	−5	30	4	11	−2	216	100	152	27	—
119	26	4	23	8	14	9	124	69	91	32	—
120	45	4	37	15	27	8	142	67	92	17	—
51	15	−4	12	5	8	—	47	29	35	22	—
88	49	−2	28	12	18	—	222	104	175	51	—
106	21	5	20	8	13	5	116	74	92	32	0.97
58	16	−1	15	5	10	3	52	36	42	25	0.73
83	20	2	19	12	14	6	89	60	72	31	0.76
100	41	2	41	13	25	6	264	148	197	53	0.98
78	53	−1	28	12	—	5	163	100	130	46	0.82
93	33	7	33	13	21	10	177	89	123	41	—
104	33	11	51	19	31	11	131	64	92	21	—
117	52	13	31	28	34	—	124	57	87	16	—
87	32	6	32	13	21	—	161	91	117	41	—
54	18	2	18	7	—	2	72	42	51	25	—
66	25	2	25	9	—	—	147	60	105	49	—
89	36	7	36	14	21	—	183	99	132	48	0.80
63	26	4	26	11	15	—	89	61	74	38	0.62
73	34	8	34	15	21	—	141	88	107	50	0.74
73	29	4	29	14	—	—	171	102	123	59	0.66
103	60	7	88	19	53	—	288	119	187	59	0.82
106	29	9	27	12	19	—	112	66	86	22	—
119	31	9	31	12	23	—	82	46	61	13	—
117	27	6	26	10	18	—	100	58	78	18	—
98	13	−1	14	3	7	—	72	41	52	16	—
118	32	1	24	5	12	—	141	72	105	22	—
102	36	0	29	6	14	—	170	87	123	24	—
98	27	8	26	11	17	13	119	76	93	27	0.92
67	16	1	13	4	9	4	71	50	60	29	0.71
100	23	6	22	10	15	7	116	74	93	27	0.82
97	34	3	31	10	19	—	233	127	169	40	1.08
91	31	3	27	9	15	6	158	96	126	35	—

Table II Data obtained in connection with heart catheterization of 4 patients with postural hypotension

Case no.	Head-up tilting degrees	Work load kpm/min.	Norepinephrine (NE) μg/min. g salt − g	Pulse rate beats/min.	Oxygen uptake ml/min.	O_2-capacity ml/100 ml	O_2-sat. %		AV-O_2 diff. ml/l	Card. output l/min.
							Brachial artery	Pulmonary artery		
1	0	Rest	—	73	284	16.2	99	76	40	7.2
	45	Rest	—	88	225	16.3	98	69	48	4.7
	45	Rest	NE 4 0	97	305	17.3	97	79	32	9.7
2 a	0	Rest	—	53	250	18.3	94	71	43	5.8
	0	500	—	90	1,268	18.8	93	32	116	11.0
	45	Rest	—	64	260	17.3	96	51	80	3.3
	45	Rest	NE 1.5	79	3 0	18 7	96	68	53	7.0
2 b	0	Rest	—	56	287	16.6	97	68	49	5.9
	30	Rest	—	60	255	16.3	98	54	74	3.5
	30	Rest	g	56	284	16.9	98	64	60	4.8
	30	Rest	NE 3 4	69	338	17 7	96	71	45	7.5
	30	Rest	NE 1.9	74	307	17.2	97	67	53	5.8
3 a	0	Rest	—	57	246	16.0	95	67	46	5.3
	0	150	—	75	696	17.2	96	45	89	7.8
	0	300	—	84	1 001	17.3	95	37	102	9.8
	0	Rest	—	58	252	16.4	96	67	50	5.1
	30	Rest	—	66	262	16.5	97	54	74	3.6
	30	Rest	NE 1.5	63	240	16.9	96	60	63	3 8
3 b	0	Rest	—	54	237	15 7	99	69	49	4.8
	30	Rest	—	53	221	15 7	98	58	64	3.5
	30	Rest	g	52	235	16.0	97	61	61	3.8
	30	Rest	NE 1.9	51	241	16.5	96	58	65	3.7
	30	Rest	NE 3 4	54	263	16.6	99	71	47	5.6
4 a	0	Rest	—	64	231	11.5	100	72	34	6.8
	0	100	—	69	494	11 7	100	50	60	8.2
	0	Rest	—	62	257	11.2	98	68	35	7.3
	40	Rest	—	58	246	11.3	99	62	44	5.6
	40	Rest	NE 1 4	73	288	11.5	98	69	35	8.1
	40	Rest	NE 2.6	88	280	12.2	98	75	31	9.0
4 b	0	Rest	—	64	209	12.2	97	70	34	6.1
	40	Rest	—	55	191	12.2	98	57	52	3.7
	40	Rest	g	62	223	12.6	96	68	36	6.2
	40	Rest	NE 1.9	77	275	13 4	99	73	37	7.5
	40	Rest	NE 0.9	70	251	13.5	95	67	39	6.4

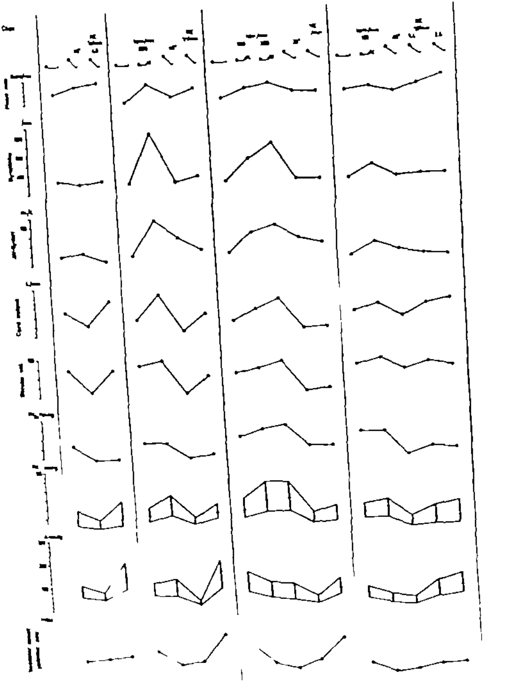


Fig. 2. Hemodynamic changes at rest and during exercise in supine position, and in head-up tilted position before and during infusion of norepinephrine. Below the number of the cases is denoted the body position, the work load in kpm/min., the degree of tilting and the rate of infusion of norepinephrine (NE) in µg/min. [illegible] mm. RV ED = right ventricle end-diastolic, PA = pulmonary artery, Br.A. = brachial artery.

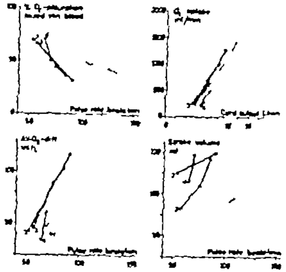


Fig 1 Some hemodynamic relationships at rest (●) and during exercise (○) in recumbent position observed in four patients with postural hypotension The dashed lines represent mean values at rest and during exercise in supine position in 9 healthy old men (12)

in healthy old men (12) The hemoglobin concentration was low in case 4 and in the other cases it was at the lower border of the variation found in normal old men (12) The *blood volume* per kg body weight in cases 1 3 and 4 was larger than the average found for normal old men It appeared to be normal in case 2 and in case 4 on the second investigation In case 1 the blood volume was almost twice as large as normal. The *heart volume* in the prone position was somewhat large in cases 2, 3 and 4 The case with the largest blood volume had the smallest heart volume.

Data obtained in connection with right heart catheterization

Rest in supine position (figs 1 2 and table II) The oxygen uptake was between + 8 and + 54 % higher than the predicted basal value (16) i. e. within ranges usually found during heart catheterization. The pulse rates were approximately the same as at rest prior to heart catheterization The arterial oxygen saturation was normal in all cases. The oxygen saturation of mixed venous blood and the arterio-venous oxygen difference were within the range of normal variation. Cardiac output with regard to age (12) was found normal in all cases in relation to the oxygen uptake (fig 1) The stroke volume was somewhat larger than the average found in old healthy men with about the same body size (12) The central blood volume varied between 0.80 and 0 97 l, corresponding to approximately 14 to 17 % of the total blood volume. The pressures in the aorta, pulmonary arterial wedge position pulmonary artery and right ventricle were normal in cases 1 2 and 4 with regard to age (12) and the reference level used In case 3 the aortic pressure was slightly high but all the other pressure determinations on the case could be regarded as normal.

Exercise in supine position (figs. 1 and 2) In three cases the effect of exercise was studied The heart rate during exercise was lower in all cases in relation to the oxygen uptake than the average for normal old men (12) The oxygen uptake during work corresponded to mechanical efficiencies between 16 and 23 % these values being normal for the work loads performed The oxygen saturation of mixed venous blood decreased and the arterio-venous oxygen difference increased faster than ordinarily in relation to pulse rate (fig 1) This is attributable in these cases to the deficient rise in pulse rate during work as compared to that of healthy old men (12) The cardiac output showed a normal increase in relation to oxygen uptake (fig 1) which means that the stroke volume increased.

The pressures in the lesser circulation increased normally during work. The

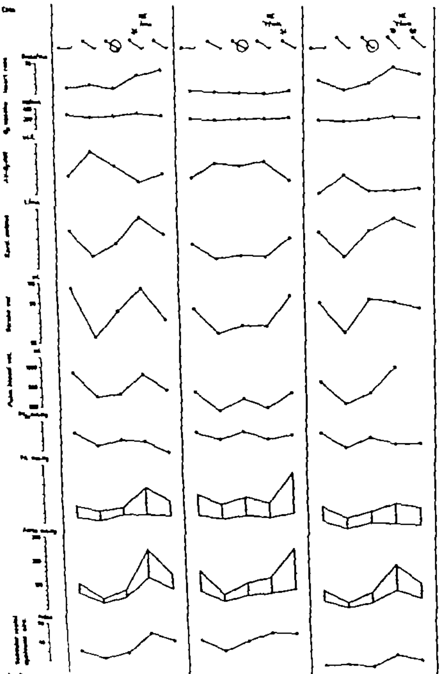


Fig. 4. Hemodynamic changes at rest in supine position and at rest in head-up tilted position before and after inflation of an antigravity suit and during infusion of norepinephrine   two different rates. Inflation of the antigravity suit is symbolized by   circle surrounding the lower half of the body. Other symbols as in fig. 2.

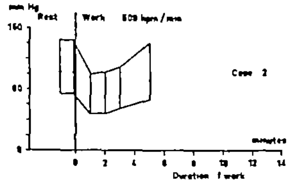


Fig 3 Pressures in the brachial artery at rest and during work in recumbent position.

systemic arterial pressure decreased markedly on transition from rest to work in all cases and remained low in cases 3 and 4 during the whole exercise period (fig 3) Thus the total peripheral vascular resistance showed an abnormally steep decrease during work.

Head-up tilted position (figs 2 and 4) In the elevated position the pulse rate increased in case 1 but remained almost unchanged in the other cases. The oxygen uptake showed only small changes The stroke volume in the tilted position was 14—54 % lower than in the horizontal position. This difference is on the average larger than that between recumbent and sitting position found in healthy old men (12) The difference in pulse rate between the tilted and horizontal positions was of the same order as that found between the sitting and lying positions in normal old men. The difference in cardiac output between the horizontal and the elevated positions was considerably larger in the patients with postural hypotension than in normal old men studied in the lying and sitting positions. The cardiac output decreased by 1.3 to 2.5 l/min a decrease of 27 to 43 % from the value in horizontal position. On changing from supine to sitting position the cardiac output decreased by 2 l/min (25 %) in normal young men (2) and by 0 6 l/min (10 %) in healthy old men (12)

The end-diastolic pressure of the right ventricle and the central blood volume decreased on head-up tilting The pressure in the aorta decreased markedly in all cases moreover the pressures in the lesser circulation decreased in all cases. The total peripheral vascular resistance decreased or was almost unchanged

Antigravity suit inflation in the head-up tilted position (fig 4) The oxygen uptake and the pulse rate showed only small changes. The filling pressure of the right ventricle the central blood volume, stroke volume and cardiac output increased to varying degrees in all cases. Both the aortic pressure and the pressures in the lesser circulation increased The total peripheral vascular resistance increased in case 2 and 3 but was little changed in case 4

Norepinephrine infusion in the head-up tilted position (fig 4) Infusion of norepinephrine in the smaller dose (0 9—1 9 μg/min) caused some increase of the heart rate

position. An impaired adjustment of the capacity vessels is not expected to influence the circulation during work in recumbent position while the legs are elevated above the heart. In the upright position, however this faulty regulation of these vessels will be consequential. An increase in the blood volume will at least to some extent compensate for a low tone of the capacity vessels. Because of the tendency to faint in upright position the patients could not be studied during work while sitting on the bicycle. The exertional hypotension is obviously more marked in the sitting position. The limiting factor in performance of muscular work seems to be the hypotension which results in fainting before the maximal cardiac output is reached. Probably these patients also have a low maximal heart rate.

When the patients were tilted to a 30°—45 head-up position the systemic arterial pressure decreased markedly This was due to an insufficient regulation of both the cardiac output and the total peripheral resistance. Thus the heart rate was almost unchanged and the stroke volume decreased more than in healthy old men (12) This resulted in a marked decrease in cardiac output, amounting to 34 % of the value in the supine position. In normal subjects the total peripheral vascular resistance increases on changing from supine to sitting position (2, 12) but in our patients it decreased or was almost unchanged.

Guyton (13) has stressed the importance of the sympathetic control of the veins for the normal regulation of the cardiac output. On tilting to an erect position a reflex venoconstriction has been demonstrated in normal subjects (25 27) In patients with postural hypotension this venomotor activity is weakened (25) The marked decrease of the stroke volume in the tilted position, following the changes of the central blood volume (fig 4) can be explained by this impaired adjustment of the capacity vessels.

It may be expected that infusion of the sympathetic transmittor substance, norepinephrine, should counteract the abnormal circulatory response to tilting in these patients. Therapeutic attempts with various pressor substances have given varying results. Bradbury and Eggleston (4) found considerable postural fall of the blood pressure even after epinephrine The same observation was made by Crost and Friedlander (5) after neosynephrine and l-norepinephrine. Luft and von Euler (22) found that norepinephrine had a favorable effect on the blood pressure in the erect position. Bickelman et al. (3) made the same observation after metaraminol. Although varying hemodynamic effects of administration of norepinephrine to normal subjects have been reported it seems well established that norepinephrine increases the total peripheral vascular resistance and scarcely influences the cardiac output (7 11) A venoconstrictor effect has also been demonstrated (27)

In the present study even a small dose of norepinephrine (0 9—1.9 µg/min) infused while the patient was tilted caused a rise in mean systemic arterial pressure to a level above the control values in the supine position. The sensitivity to norepinephrine is considerable in these patients. The abovementioned doses correspond to 0.015—0.031 µg/kg/min. According to Goldenberg et al. (11) the minimum amount of norepinephrine required to produce an increase of blood pressure was on the average 0 05 µg/kg/min in normal subjects. The increased sensitivity to norepinephrine in these pa

and oxygen uptake in cases 2 and 4. In case 3 the changes were insignificant. The stroke volume and the cardiac output increased in all cases. In cases 2 and 4 these effects were marked and the cardiac output reached or surpassed the value at rest in the horizontal position. In case 4 the stroke volume was also restored to the supine control value. The central blood volume increased slightly when compared to the control value in the tilted position. With a larger dose of norepinephrine this increase was more marked. The end-diastolic pressure of the right ventricle showed small varying changes. Systemic arterial pressure rose promptly to a value similar to or above that at rest in the horizontal position. The pressures in the pulmonary artery and right ventricle increased to approximately the control levels in the lying position. Total peripheral vascular resistance was higher than at rest in the supine position. With a larger dose of norepinephrine (1.9—4.0 μg/min.) the changes described above were more pronounced. This was especially true for the systemic arterial pressure which rose to abnormally high values.

Discussion

Normally the systemic arterial pressure is very delicately adjusted (17). The regulation operates with the baroreceptors as the tactile organ. It is arranged as a counter-coupled feed back system balancing via the sympathetic outflow the total peripheral resistance and the cardiac output. The cardiac output can be controlled by adjustment of the venous capacity vessels which according to Folkow (9) can dramatically affect the filling of the pump. Cardiac output can also be controlled by direct sympathetic influence on the contractility and rate of the heart. An increase in heart rate is fairly ineffective, however, if ventricular filling is impaired. In cases with postural hypotension these regulatory mechanisms are more or less destroyed, possibly due to a central lesion (32). This disturbance will be evident in the erect position and especially during exercise.

In healthy individuals the systemic arterial pressure may decrease slightly during the first 10—15 seconds of exercise but then increases to a higher level than at rest. The initial pressure fall is more constant and marked in the sitting than in the lying position (21). von Euler and Liljestrand (8) found a decrease in the systemic arterial pressure in animals during electrically induced work when the pressoreceptors had been denervated. In postural hypotension Marshall et al. (23) also reported an exertional hypotension during work in the reclining position. Our three patients studied during work in the recumbent posture similarly showed a marked decrease in systemic arterial pressure. The pressures at rest, indicated in fig. 3, were measured immediately before work when the patient had the feet elevated on the pedals of the bicycle, but the pressures at rest, indicated in fig. 2, were recorded some minutes before work was begun (legs held in the horizontal position). The cardiac output which was normal at rest increased normally during exercise. The vasodilatation, elicited in the active muscles during work, is obviously not compensated for by reflex vasoconstriction in other regions of the vascular bed and therefore results in an abnormally steep decrease in the total peripheral vascular resistance. The increase in cardiac output was to some extent due to an increase in the stroke volume. This indicates an adequate ventricular filling during exercise in the supine

4. On changing from recumbency to a head-up tilted position the systemic arterial pressure fell markedly but the heart rate was little changed. The cardiac output and the stroke volume decreased abnormally when compared to healthy old men of about the same body size. The results indicate impaired adjustment both of the resistance vessels and of the capacity vessels.

5. Infusion of a small dose of the sympathetic transmitter substance, norepinephrine, when the patient was tilted, restored blood pressure and cardiac output to about the same values as in the supine position. The end-diastolic pressure of the right ventricle did not show any definite relation to the change in stroke volume. With infusion of larger doses of norepinephrine the blood pressure increased abnormally. The stroke volume then reached the same value as in the supine position and the cardiac output exceeded the control value in the recumbent position. Actions on resistance and capacity vessels and on myocardial contractile force explain these effects of norepinephrine.

6. Pressure applied to the lower half of the body by means of an antigravity suit, worn while in the tilted position, partly restored blood pressure and caused an increase of the stroke volume and cardiac output. The effect was similar to that of a small dose of norepinephrine and may indicate that an abnormally large displacement of blood to the periphery is partly responsible for the circulatory disturbance in the tilted position.

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tients with postural hypotension is compatible with a low sympathetic tone The observation that atropine does not increase the heart rate in patients with postural hypotension (4 5) may also indicate a low sympathetic tone. In spite of the fact that systemic arterial pressure rose above the value in supine position the heart rate increased In normal subjects there is usually a reflex decrease in heart rate when systemic arterial pressure rises after norepinephrine (11) This abnormal reaction in patients with postural hypotension also indicates that the normal reflex pressor response is abolished.

Even with a small dose of norepinephrine the cardiac output was restored to the average control value in the supine position The central blood volume tended to increase. The stroke volume also increased but did not reach the same value as in the supine position. The end diastolic pressure of the right ventricle did not show any definite relation to the increase in stroke volume possibly because of altered myocardial contractility

The increase of the systemic arterial pressure can be explained both by arteriolar constriction and the observed rise in cardiac output The increase in cardiac output is probably accomplished by adjustment of the venous capacity vessels as well as by cardiotropic influence. With norepinephrine an increased venous tone has been demonstrated by Sharpey Schafer (27) and an increased myocardial contractile force by Goldberg et al. (10)

Previous studies have shown that pressure applied to the lower half of the body by immersion in water (30) or by the use of a counter pressure garment (28) can in some cases, partly prevent the postural decrease of blood pressure. This effect may be lacking in other cases of this type (5) In the present study inflation of an antigravity suit, worn by the patient partly restored the systemic arterial pressure and prevented syncope while in the erect position. Under these circumstances heart rate remained unchanged Right ventricular filling pressure stroke volume and cardiac output increased The effect was similar to that of infusion of a small dose of norepinephrine although the total peripheral vascular resistance did not change to the same extent. This favorable effect of the antigravity suit indicates that an abnormally large displacement of blood to the periphery may be partly responsible for the circulatory disturbance in the erect position.

Summary

1 Four patients with postural hypotension have been investigated with right heart catheterization. The total hemoglobin blood volume, heart volume and exercise tolerance in the supine position were previously determined.

2 The circulatory response to recumbent exercise and head-up tilting was studied Observations on the effect of norepinephrine infusion and antigravity suit inflation in the tilted position have been included

3 In the supine position the cardiac output was normal in relation to the oxygen uptake both at rest and during work. There were no signs of impaired ventricular filling in the supine position. The systemic blood pressure decreased abnormally during recumbent exercise due to impaired reflex control of the resistance vessels. The exertional hypotension which is obviously more marked in these cases in the sitting position, seems to be the factor which limits performance of muscular work.

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The Antiproteolytic Action of some Antacids

By

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It is generally agreed that the final event in the pathogenesis of duodenal and gastric ulcers consists of digestion of the mucosal cells by proteolytic enzymes, notably pepsin, in the gastric juice. However it is not known, whether the hydrochloric acid causes mucosal damage by a more direct action than by activating proteolysis. Antacid preparations are thought to act beneficially in the therapy of peptic ulcer by reducing the hydrogen ion concentration, thereby also reducing the proteolytic activity of the gastric juice. However it has been pointed out that gastric proteolysis, at least on certain substrates, is relatively rapid at pH levels (pH 3—5) prevailing during therapy with modern antacids (2, 3). It is therefore possible that the measurement of intragastric pH alone is an unreliable means of assaying the therapeutic efficacy of an antacid preparation. Ideally one would like to measure the influence of an antacid preparation on the total damaging action of the stomach contents upon the mucosal cells.

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Recent work (3) has demonstrated that some substances, suitable for use as antacid preparations, have an antiproteolytic activity *in vitro* apart from that caused by their effect on the pH. The present paper describes *in vitro* and *in vivo* experiments on the effect of some of these substances on the proteolytic activity of human gastric juice, obtained by insulin stimulation.

Experimental

Experiments *in vitro* were performed essentially according to Persson & Bunke (3). The modifications introduced were: The unfiltered gastric juice was prepared for incubation by gentle homogenization in the cold in a Potter Elvehjem homogenizer. The antacid was added to the undiluted gastric juice (10 ml) as a suspension (350 mg antacid in 1.5 ml of 0.17 % tragacanth solution) by means of a syringe. After exactly 5 min. of preincubation, the mixture was centrifuged. The upper part of the supernatant was used for the determination of the proteolytic activity after suitable dilution. The normal value was determined correspondingly. En-

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Table I. Working schedule and results of an [illegible] experiment. The onset of the insulin-stimulated secretion was taken as zero time. The stomach was emptied at each time interval, which is important to note as variable amounts of the added antacid were aspirated together with the juice. Therefore the results in this table give no information concerning duration. The antacid was given as suspension: 1 g antacid in 5 ml of tragacanth solution (170 mg tragacanth/100 ml water). The volume and the enzyme concentration of the juice collected the first time after the antacid addition were adequately corrected for dilution. The enzyme activity was always measured at pH 4.0. Immediately before addition of the antacid the activity was 9.2 mg tyrosine/ml/min. The activity in the other samples are expressed as per cent of this activity. Antacid: Aluminum hydroxide-magnesium carbonate (co-dried gel)

Time in minutes	Ml of gastric juice obtained	pH	Relative enzyme conc. (per cent)
5	10.5	1.38	128
10	11.5	1.13	113
15	10.5	1.03	104
20	11.5	1.04	100
23	1 g antacid added		
30	9	4.20	25
35	11	2.80	47
40	7	2.20	84
45	4.5	1.20	125
50	7	1.50	103

showed that the stomach was still secreting efficiently in response to insulin. The pH increase was about the same with dimagnesium aluminum trisilicate as with the co-dried gel, but the inhibition of proteolysis was smaller. Magnesium trisilicate did not increase the pH above 2 and caused no inhibition of proteolytic activity. It should be noted that the proteolytic activity was measured at the same pH (pH 4) throughout the experiments; therefore the inhibition of proteolysis seen during the first periods after giving the antacid is an effect apart from the effect on the pH of the gastric juice.

A summary of the inhibition of gastric

Table II. Inhibitory effect of some antacids on the proteolytic activity of insulin-stimulated gastric secretion. Compare table I

Antacid	Experimental subject no.	Gastric juice obtained 5-7 min. after the addition of antacid: pH	relative proteolytic activity (per cent)
Aluminum hydroxide-magnesium carbonate (co-dried gel)	I	4.13	22
	II	4.20	26
	III	3.98	32
	IV	4.01	42
	IV	4.20	25
Di-magnesium aluminum trisilicate	I	3.81	67
	II	3.85	34
	III	3.78	76
	IV	3.95	100
	IV	4.12	55
Magnesium trisilicate	I	1.55	91
	II	1.48	88
	III	1.60	111

proteolysis by three of the substances previously examined *in vitro* (3) is given in table II which shows the proteolytic activity expressed as per cent of the activity in the specimen of juice collected immediately before the injection of the antacid.

Discussion

The present experiments confirm and extend the previous findings *in vitro* on the inhibitory action of some antacid substances on commercial pepsin preparations (3). Of the substances examined, aluminum hydroxide-magnesium carbonate (co-dried gel) was the most effective inhibitor of proteolytic enzymes present in human gastric juice and the inhibition could be obtained both *in vitro* and by adding the antacid *in vivo* and then measuring the rate of proteolysis in samples of gastric juice removed from the

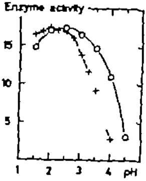


Fig 1

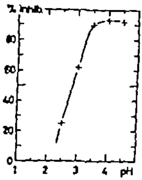


Fig 2

Fig 1 Relative proteolytic activity of a commercial pepsi preparation ( ) and human gastric juice (—) as functions of pH The curve of the gastric juice represents the mean value of five curves obtained with juice from five different persons. The forms of these curves were identical. Substrate 1 6 (w/v) human CO-hemoglobin. Temp 37° C. The enzyme sol tions were diluted with 0 1 M NaCl containing 0 01 mole KCl/l, before the determination of the activity

Fig 2 Per cent inh bition of the proteolytic ctivity of human gastric juice (undiluted) by aluminum hydroxide-magnesium carbonate (co-dried gel) as a function of pre incubation pH Antacid concentration 30 mg/ml Calculated value of standard enzym activity at pH 3 1 (substrate CO-hemoglobin, temp. 37 0 C) 7.6 mg tyrosine liberated/ml/min. Enzyme ctivity/antacid concentration ratio: 0.25. Time for preincubation with ntacid 5 min.

zyme activity was expressed as mg tyrosine liberated from human CO-hemoglobin per min by 1 ml of gastric ju ce.

Experiments *in vivo* were made on healthy male volunteers after twelve hours of fasting After introduction of a stomach tube 16 i. u. of insulin were injected subcutaneously The gastric juice was collected n 5 min. periods by almost constant manual suction with a syringe. The rate of secretion was about 0 5—1 ml gastric juice per min. before the onset of insulin stimulation. When the secretion was well on the increase, in response to the nsulin, the antacid substance was injected as a suspension through the tube. The gastric juice obtained was measured and centrifuged immediately The supernatants were put on ice until the pH was measured 1—2 hours later The proteolytic activity was then measured after adjustment of the pH to 4. The working schedule is shown in table I

The measurements of proteolytic activity were performed after suitable dilution (1 part of gastric juice to 200 or 300 parts of 0.1 M NaCl containing 0.01 mole of KCl per litre) according to the modified method of Anson & Mirsky (1 3) The pH of the centrifuged gastric juice was measured with an ordinary pH-meter

The following antacids were examined aluminum hydroxide magnesium carbonate (co-dried gel, Rehets Co., New Jersey), dimagnesium aluminum trisilicate and mag nesium trisilicate. Other materials used were the same as described elsewhere (3)

Results

It was first necessary to study the relation between pH and proteolytic activity in human gastric juice with the prevailing experimental conditions. Fig 1 shows the results in comparison with those obtained with a commercial pepsin preparation. The different behaviour of human gastric juice and pepsin preparation made it necessary to study the relation between pH and % inhibition of proteolytic activity of human gastric juice. Fig 2 shows this relationship for the most efficient inhibitor aluminum hydroxide magnesium carbonate (co-dried gel) The curve obtained was nearly identical with that previously observed for the same substance on commercial pepsin preparations.

It was found that the addition of aluminum hydroxide magnesium carbonate (co-dried gel) *in vivo* caused an immediate increase in pH of the gastric juice to a level of about 4 In addition the proteolytic activity was diminished. A typical experiment is shown in detail in table I Later on, as the antacid concentration was reduced by removal through the stomach tube both acidity and proteolytic activity increased again, which

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The Effect of Nicotinic Acid on the Plasma Free Fatty Acids

Demonstration of a Metabolic Type of Sympathicolysis

By

Lars A. Carlson and Lars Orö

The demonstration of Altschul et al. in 1955 (1) that nicotinic acid had a cholesterol lowering effect in man, has attracted great interest from the experimental clinical side as well as from the biochemical point of view. Experience of long term treatment of human beings with nicotinic acid has been reported e.g. by Parsons (2) and all reports have shown permanent depression of the plasma cholesterol by nicotinic acid. Furthermore, it has been shown that the plasma total lipids and total esterified fatty acids also decrease during nicotinic acid treatment (3 4). Cutaneous xanthomata such as xanthoma tuberosum have been reported to disappear in several cases (2). These findings indicate that nicotinic acid has a major action not only on plasma cholesterol but also on elevated plasma glyceride levels. The mechanism for this action of nicotinic acid is not known and a great number of hypotheses have been put forward, including decreased cholesterol synthesis as well as an increased cholesterol catabolism.

During recent years more insight has been gained about the importance of plasma free fatty acids as precursors of the plasma glycerides and other plasma lipids (5 6, 7 8 9). The acute effect of nicotinic acid on the plasma FFA fraction has not been studied previously. The results from such studies in man are reported here. The findings observed after administration of nicotinic acid initiated some studies on the effect of nicotinic acid on the norepinephrine induced increase of FFA which are also reported.

Material and methods

Subjects

Three volunteers, two males and one female were studied. They all had moderate elevation of plasma cholesterol and triglycerides, but there was no evidence of any other metabolic disease. They had fasted overnight. During the investigation they were lying down. All blood samples were taken from a small tephlon catheter inserted into the brachial artery. There was an initial control period during which four blood samples were taken at 15 min. intervals. The nicotinic acid was

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stomach. The concentration of antacid necessary for obtaining a good inhibition was of the same order as that suitable for ulcer therapy. The second substance investigated, dimagnesium aluminum trisilicate, seems to be a less active inhibitor of proteolysis. This is in accordance with the previous *in vitro* results (3). The third substance, magnesium trisilicate, did not increase the pH above 2 in the present experiments. The lack of an inhibiting action on proteolysis is in agreement with earlier results (3).

More work is needed to establish the degree of inhibition of proteolysis actually obtained in the stomach. It would be especially interesting to study the effect of antacids on the destructive action of gastric juice on mucosal cells, blood clots and other "natural" substrates of relevance for the genesis and therapy of peptic ulcer.

Summary

Some substances suitable for the use as antacids in ulcer therapy were examined as to their effect on proteolysis in human gastric juice *in vitro* and *in vivo*. An inhibition of proteolysis was observed apart from that caused by raising the pH.

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Fig. 2. Effect of infusion of norepinephrine on arterial FFA level and on blood pressure in two anesthetized dogs. Norepinephrine was given as indicated by the black bars.



Fig. 3. Effect of nicotinic acid on the blood pressure and arterial FFA response to norepinephrine in three anesthetized dogs. Nicotinic acid was given intravenously at the arrows. Norepinephrine was infused as indicated by the black bars.

Discussion

In man nicotinic acid exerted a definite, but short FFA depression. The data from these experiments do not allow any conclusion as to the cause of this effect. An increased outflow of FFA from plasma as well as a decreased inflow from adipose tissue, the main source of FFA, are theoretically equally possible. However the flow of FFA from adipose tissue appears to be the main factor regulating the plasma FFA level (e.g. 10). Thus, factors and conditions increasing plasma FFA as catecholamines, glucagon, fasting and diabetes all increase the output of FFA from adipose tissue. On the other hand, glucose and insulin which decrease the plasma FFA level also decrease the output of FFA from the adipose tissue. On the other side it ought to be mentioned that the outflow of FFA from plasma might be related to the amount and the distribution of the blood flow. An increased blood flow through muscular tissues as observed during exercise could thus give rise to an increased outflow of FFA from plasma (12, 13, 14). Nicotinic acid is not known to effect the distribution of the blood flow except by the cutaneous capillary vasodilation. We did not observe any change in pulse rate or blood pressure in our experiments in man. The most probable explanation for the decreasing effect of nicotinic acid on FFA would thus appear to be a diminished delivery to plasma from the adipose tissue.

This reasoning led us to study the effect of nicotinic acid on FFA release from adipose tissue. Among other things the effect on the norepinephrine induced FFA release was investigated, i.a. since norepinephrine is one of the most potent stimulators of FFA release. The results clearly demonstrate that nicotinic acid has a metabolic type of sympathicolytic effect not demonstrated previously with a blocking of the norepinephrine effect on FFA release while the pressor response is unchanged. The possibility of dissociating the metabolic and pressor effects of norepinephrine will be studied later.

A direct relationship between the reduction of the basal FFA level after nico-



Fig 1 Effect of nicotinic acid on arterial plasma FFA level in three human beings. At the first arrow 200 mg of nicotinic acid was given by mouth and at two other arrows 100 mg was given.

then given by mouth, first 200 mg, followed by two 100 mg doses one and two hours later Blood pressure and pulse rate were not influenced by this dose of nicotinic acid.

Animal experiments

Five dogs weighing 14—24 kg were used They had fasted overnight and were anaesthetized with Nembutal (Abbot) intravenously Blood pressure was measured with an Elema-Schönander pressure transducer (EMT 490 A) from a tephlon catheter inserted into the femoral artery The blood samples were also taken from this artery The pressure transducer was filled with saline and no heparin was injected into the animal. Norepinephrine (l base) was infused intravenously at a constant rate of 0 6 µg/kg/min. during two twenty minute periods. Between these periods, in three experiments, nicotinic acid was given in a total dose of 100 mg/kg body weight, divided into ten equal doses. The nicotinic acid was administered intravenously over a period of one hour during the first half hour at 10 min. interval and during the second at 5 min. interval. After the last dose of nicotinic acid the second norepinephrine infusion period was started. Two dogs were used as controls, and in this case no nicotinic acid was given between the two norepinephrine periods.

Analytical methods

In all experiments the arterial blood samples were taken into heparinized tubes and centrifuged immediately and the plasma free fatty acids were determined according to Dole (10). Addition in vitro of nicotinic acid did not influence the titration values.

Results

Effect of nicotinic acid on plasma FFA in man

The acute effect of nicotinic acid in the three studied subjects is given in fig 1 It is seen that during the control period the FFA remained constant. About 15—30 min. after the administration of nicotinic acid the FFA level decreased This occurred simultaneously with the appearance of the flush After about 60—90 min. the FFA increased again. There then occurred a rise of the FFA above the basal level.

Effect of nicotinic acid on the norepinephrine induced FFA release in dog

The effect of two infusions of norepinephrine on blood pressure and plasma FFA in the anaesthetized dog is given in fig 2 The figure demonstrated that the response in the dogs to the second norepinephrine infusion was qualitatively and quantitatively similar to the response after the first infusion with regard to blood pressure as well as FFA.

After administration of nicotinic acid, however the increase of plasma FFA after norephinephrine administration was almost completely abolished as demonstrated in fig 3 This figure furthermore demonstrates that the effect on the blood pressure was unchanged after nicotinic acid

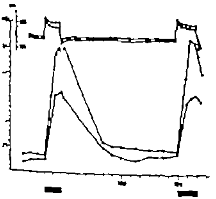


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A direct relationship between the reduction of the basal FFA level after nico-

tinic acid by mouth and the inhibition of FFA release by norepinephrine has not been demonstrated here but such a relationship appears likely It has thus been suggested that the sympathetic nervous system exerts a tonic effect on the adipose tissue (16) and the release of FFA from this tissue. Norepinephrine has also been shown to be present in adipose tissue (17) The effect of nicotinic acid might thus well be due to a metabolic sympathicolysis in adipose tissue. The overrise phenomenon observed might in that case be related to a compensatory increased sympathetic tone in adipose tissue.

It does not appear likely that the decrease of FFA would be due to an increased carbohydrate utilization in the adipose tissue as far as could be judged from most data in the literature. Conflicting results are, however reported in the literature about the effect of nicotinic acid on glucose metabolism. Lammers et al (17) found a delayed glucose elimination in rat and also after long term administration in man a decreased carbohydrate tolerance has been found (3) On the other hand Mirsky et al (18) reported a glucose depressing effect of nicotinic acid in rats.

We have studied the effect of nicotinic acid in two other volunteers and found no significant changes on the blood glucose while the usual fall in FFA was observed (unpubl. results) Furthermore the administration of insulin and glucose before infusion of norepinephrine does not abolish the FFA response elicited by norepinephrine (20)

A direct relationship between this acute effect of nicotinic acid and the long term plasma lipid depressant effect has, of course, not been established in this work. From the theoretical point of view however there might well be a cause and effect relationship as indicated in the introduction. Furthermore, it has previously been shown from this laboratory that chlorpropamide in acute experiments reduces the plasma FFA (21) and during long term treatment has reduced the plasma glycerides (22) To mention another type of drug salicylates have been shown to decrease the plasma cholesterol levels (23) and in acute experiments with salicylates the plasma FFA level is reduced (24) A fact which strongly suggests that there is a direct relationship between the acute FFA lowering effect and the prolonged plasma lipid reduction of nicotinic acid is that nicotinamid does not appear to reduce the FFA level in acute experiments (25) Nicotinamide does not reduce the plasma lipid level during long term treatment. It is tempting to suggest, that a general principle worthy of further investigation is that a decreased release of FFA from adipose tissue will reduce the plasma lipid level in man.

Summary

In acute experiments the administration of nicotinic acid to man caused the plasma free fatty acids (FFA) to decrease quickly in order to increase soon again above the basal level

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The possibility of a close relationship between reduction of plasma FFA and a depression of the other plasma lipid fractions is discussed.

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Ulcerative Colitis

A Follow-up Investigation of a 20-year Primary Material

By

R. Carlsson, B. Fernstedt and J. Philipson

The casuistics that have been published in the field of ulcerative colitis are *as a rule difficult to compare* and, as has been said on various occasions, it is difficult to assess statistical data concerning the cause of disease and the mortality. The material has been selected and treated in various ways.

Most of it originates from special clinics or from hospitals where there is a special interest in this disease or where a special method of treatment is applied. In such material there is often an accumulation of serious cases which might give an erroneous impression of the prognosis of the disease. It should therefore be valuable to have at hand a random sample from the general population. We have made a follow-up examination of patients who, during a period of 20 years, have been admitted to a hospital within a defined district (approx. 180,000 inhabitants). The material includes all cases of ulcerative colitis that required hospital care. Accordingly, cases of mild ulcerative proctitis and proctocolitis may have been missed and only cases with a definitive diagnosis of ulcerative colitis have been included. The figures may therefore be rather an underestimate.

Material

The material comprises those patients who during the 20-year period 1939—1958 were admitted to the Örebro Centrallasarett (Örebro Central Hospital) (medical, surgical, paediatric and child-psychiatric departments) or to the Örebro Epidemic Hospital with the *diagnosis of ulcerative colitis*. Only such cases were included where the diagnosis was ascertained by characteristic rectoscopic findings with visible ulcerations or by positive findings at X-ray examination. All cases where ulcerative colitis has not been definitely determined have been excluded. Follow-up examinations were made in the autumn of 1959 and the spring of 1960. During the time mentioned, altogether 98 patients with ulcerative colitis were admitted for hospital care. This number represents 3.5 patients per 10,000 admitted to the entire hospital and 15 patients per 10,000 admitted to the medical

Submitted for publication April 6, 1962.

Table II Fate of patients who were followed up for more than 20 years

Sex	Age of onset	Years of history 0–10	10–20	20–30	Status at the follow-up
♀	5	H HH			Good
♂	7	H	HH H H		Good (11 years)
♂	10	H H H H	HH H H [I] H	HH † Ca	—
♂	16	H H H † Emaciation			—
♀	17	H H HH	H H H H	H H	Mild symptoms
♀	17	H H H	H	H	Loss of haustra
♀	18	H H H			Op. discussed
♂	19	H	H H	H [C]	Good
♀	20	HHH			Good (17 years)
♀	20		—	— H (†)	—
♂	21	H H † Emaciation			—
♀	23	H H	H H H [C] Ca	[C] Polyps	Good (5 years)
♂	24	— H H	H H H	H H H H	Op. indicated
♀	25	H HH	H H	H [C]	Good
♀	25	HH	H H	H	Mild symptoms
♀	26	H	H		Mild symptoms
♂	31	H H			— (Good)
♀	32	H H	H H		Stricture
♂	39	HH H H H	H HH [C] H		Invalid
♀	45	H			— (Good)
♀	56	H [I] † Perforation			—

H = Hospital admission for ulcerative colitis.
[C] = Colectomy [I] = Ileostomy [R] = resection.
† = Died of ulcerative colitis, (†) = died of other cause.

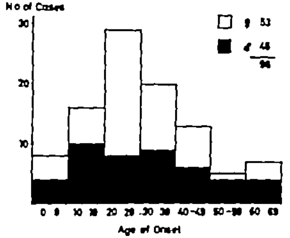


Fig 1 The composition of the material classified according to the age at the first phase of ulcerative colitis.

Table I Causes of death

		No. of cases
Ulcerative colitis		13
Acute fulminating	4	
Acute phase	3	
Emaciation	4	
Cancer of the colon	1	
Cirrhosis of the liver	1	
Other causes		2
Total		15

clinic. The proportion between the sexes was 54 % women and 46 % men.

In 91 patients out of the 98 the illness arose during this 20-year observation and in the remaining 7 the onset was some time earlier. In 54 % the disease began before the age of 30, in 24 % before the age of 20 and in only 12 % at over 50 years. There was a big scatter in the age range varying from 3 years to 68 years. In 5 of the patients the disease started acutely and fulminantly. At the time of the follow-up examination 15 of the 98 patients were dead, 13 of them having died of ulcerative colitis and its complications.

Of these 13 patients 8 died within a year after the onset of the disease and another in the second year. The age of these patients was between 35 and 66 years and 6 of them were over 58 years of age. In the remaining 4 fatal cases the duration of the disease exceeded 5 years. Four patients died of "primary-acute" fulminant ulcerative colitis, with intestinal symptoms after only a few days or weeks. One died on admission of haemorrhage and shock, the 3 remaining cases were treated medically as well as surgically (enterostomy and colectomy) and the causes of death were postoperative coma, sepsis and peritonitis. All those who died in an acute stage of the disease had had complaints for 1/2—2 years. One died of perforation and peritonitis after a few weeks treatment and two others after one week of postoperative peritonitis.

Four died from emaciation at the age of 22, 39, 40 and 66 years, having had the disease for 6, 7, 1/2 and 1 years respectively. The first two had severe changes in the entire colon and complications in various distant organs. At the autopsy embolism of the lungs was found in both cases, and moreover one of the patients had perforation peritonitis. In the latter two the course was less violent. Cirrhosis of the liver was the cause of death in a girl who got her colitis between the age of 3 and 7 years and died at the age of 17 years. In one case only the cause of death was cancer of the colon. The colitis started in that case at the age of 10 years. Ileostomy was performed at the age of 26 years and after having been free from symptoms for 6 years, the patient died of cancer of the colon with metastases.

Pneumonia and cerebral haemorrhage was the cause of death in 2 who died of a different disease. Both had remained free from bowel symptoms for several years.

The duration of the disease is apparent from fig 2. More than half or 55 % have had the disease for over 10 years and 18 % for over 20 years. Table II shows the course of the disease in patients who had had the malady for over 20 years.

Eighteen patients were operated on (table III), 9 of them in the acute stage of the disease with a mortality of 5 and the other 9 in a quiescent stage with improvement.

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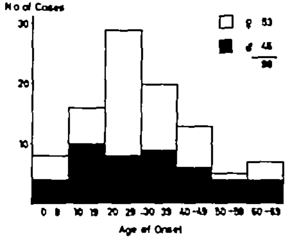


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Table II. X-ray changes at the time of the follow-up examination in relation to findings at rectosigmoidoscopy

Extent of disease	Sigmoidoscopy findings (yrs)						No. of cases
	0—4	5—9	10—14	15—19	20—24	25	
Normal colon.	●	○○○●●●	○○○○○●●□	○○	—	n	19
Rectosigmoid only	n●■	○●□	n○○●●□□□	●●	●	●	18
Up to splenic flexure	●□■	○	○	○●□	n○■	—	12
Up to hepatic flexure	□	n□	n	n	●	—	6
Entire colon.	—	●●●□	●	—	n■	—	7
Entire colon plus ileum	□	—	●	—	●	—	3
Slight stricture		□□	●	□	●□■■	—	9
Stricture	—	●	n	■	—	●	4
Pseudopolyps.	—	n●	n○●	—	n■	●	8

= No rectosigmoidoscopy 10 cases.
○ = Normal, 17 cases,
● = Inactive, 23 cases,
□ = Moderately severe, 11 cases,
■ = Severe, 4 cases.

colon in 19 cases. Abnormal roentgenological findings were noted such as flattened mucous membrane, lack of haustration with or without ulcerations, constriction of the lumen, stricture or polyps. Pronounced polyps were not apparent in any case. The extension in the intestine is indicated by the following percentage figures (table IV). Lesions affecting rectum and sigmoid colon 28 %, up to the splenic flexure 19 %, up to the hepatic flexure 9 %, in the entire colon 11 % and the entire colon with terminal ileum ("backwash ileitis") 5 %. There was no correlation between the duration of the disease and the extension of the changes in the intestine. It should be observed that the extension in the intestine here mentioned refers to the time of the follow-up examination and not to the time when the disease was at its most active stage. The rectosigmoidoscopy showed completely normal findings in 31 %, milder changes such as are usual when the colitis is healed in 43 %, slightly active colitis in 20 % and severe colitis in 7 % (table IV).

Total remission, no subjective complaints, normal findings on rectoscopy and normal colon X-ray without changes in the contours of the walls or obliterated haustration, was evident in 10 cases. Five of them had had complaints more or less continuously for some years and after that they had been free from complaints for 6, 8, 10, 10, and 19 years respectively. The X-ray changes were never pronounced and in all cases limited to the descending colon and distally thereof. In 2 similar cases that had been free from complaints for 3 and 5 years respectively, follow-up examination showed moderate loss of haustration in the sigmoid. Remission was maintained for 2 years in 2 cases who had had complaints for 11 and 12 years respectively. The last case in this group is represented by a girl born in 1951 who got the disease at the age of 3 years in the form of ulcerative colitis with changes mostly in the sigmoid. After an illness without remission for 2 years, surgery was planned. Cortisone was administered, whereupon she improved and has suffered no complaints since the autumn of 1957. Rectoscopy and X-ray of the colon in 1958 and 1960 showed no pathological changes.

Fig. 3 and 4 show *results* of the follow-up examination. 26 % of the total number of cases can be assigned to the group of deaths, colectomy due to tumours or chronic invalidity and severe symptoms of ulcerative colitis. 56 % are completely or almost free from complaints. 10 % showed total remission. Only 6 % of the survivors are quite incapacitated for work. Two cases of carcinoma of the

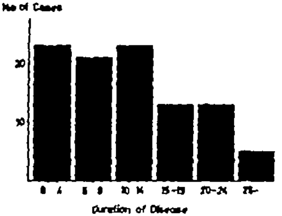


Fig 2 Duration of the disease since the onset.

Table III Indications for operation and mortality in 18 patients following surgical treatment

Indications for operation	No. of cases	Deaths	
		No.	%
Acute fulminating	4	3	56
Acute phase	5	2	
Cancer and polyps	2	0	0
Chronic ulcerative colitis	7	0	
Total	18	5	28

Four cases of "primary-acute fulminant ulcerative colitis were operated on, of which 2 were with regional localisations in the transverse colon A resection of this part of the colon was made with one postoperative death due to perforation peritonitis, while the other improved considerably In the remaining two the lesion extended over the entire colon. Colostomy was performed in one case and the patient died of sepsis after 2 weeks. In the other enterostomy-colectomy was performed and the patient died a few days after the operation. Five cases were operated on in the acute phase of chronic ulcerative colitis. All of them were in poor condition with suspect or manifest perforation peritonitis. Colectomy in one case resulted in post operative death due to peritonitis. Ileostomy and subsequent hemicolectomy was performed in another Seven years later this patient has not yet regained full working capacity In 3 cases the colon was excluded by ileostomy One of those died of peritonitis and one got the stoma closed one year later and is now 10 years after the operation still without complaints and has normal X-ray and rectosigmoidoscopy showing a normal picture. The last case improved rapidly after ileostomy Colectomy was advised but never performed because the patient did not experience any symptoms and refused operation. He died 6 years later at an age of 32 of carcinoma of the ascending colon with metastases.

Because of adenocarcinoma, in the splenic flexure, in a 41 year-old woman who had had ulcerative colitis for 18 years, a total colectomy with ileorectal anastomosis was performed. She has been in full working capacity for 10 years after the operation and she is still well. Suspected malignancy led to resection in one case where, however microscopical diagnosis gave evidence of benign polyps. Seven patients with chronic ulcerative colitis were operated on at a quiescent phase of the disease. The indications were chronic invalidity pronounced subjective complaints, polyps and stricture. Total colectomy was performed in all cases, ileostomy in 6 and ileoproctostomy in one case. The operation was performed after 1 3 8 9 11 20 and 25 years respectively of illness. No deaths occurred in this series. All of them are in good health.

Surgical treatment was considered during the follow-up in a further 4 cases due to disabling complaints and pronounced X ray changes. Three of them have now been operated on with satisfactory results.

We have been in personal touch with all the 83 surviving cases. Of the ones that were not operated on, 4 said that they had such severe complaints as to be considered incapable of work (reported above) 15 had moderate and 25 had mild periodic complaints. The remaining 30 patients reported complete freedom from symptoms for at least a couple of years. Seventy four cases had been examined during the autumn of 1959 and the spring of 1960 Those with pronounced subjective complaints were admitted to the hospital, while the rest of the patients were examined as out patients and a few were seen at other hospitals. Of the 9 who were not undergoing the examination, all considered themselves to be free from complaints and 5 of them were examined during 1958 and early in 1959 The X-ray showed a completely normal picture of the

cerative colitis is rare, but has been described (22). There is no such case in our material, but in 1959 a 30-year-old man died of "primary-acute" ulcerative colitis and half a year later his younger sister fell victim to the same disease.

We have given a separate grouping to the "primary-acute" fulminant form and the acute fulminant phase of chronic ulcerative colitis. In the first group there is an intensive destructive process in the formerly healthy tissue, and the course of the disease is grave with serious toxic reaction and high mortality rate. In the group of the acute phase the chronic inflammatory changes represent, in a way, protection against the destruction and absorption of toxic products. Of the 98 cases 5 were "primary-acute" which has been accounted for in an earlier publication (7). The age was notably high, 3 of them being over 60 years old (36, 58, 64, 65 and 65 years). The question arises whether these cases do come under a different aetiology, perhaps even a different disease from the chronic, ulcerative colitis with an intermittent course which befalls younger people. The history of the disease takes a course of 1—4 weeks. We noticed a quick succession of diverse symptoms such as diarrhoea, septic fever, deterioration of general condition due to pronounced toxic reactions, protein loss and electrolyte imbalance — especially deficiency of potassium — in our "primary-acute" form of the disease with a notable resistance to therapy. Only one of the 5 cases survived. In this case it was a question of regional colitis in the left part of the transverse colon and a resection was performed at an early stage. Kellock (14) has described 8 cases with acute segmental ulcerative colitis and usually with localisation in the transverse colon close to the splenic flexure. During the period 1959—1960 another 3 cases with "primary-acute" fulminant ulcerative colitis were admitted. All three died postoperatively, which further emphasizes the peculiarities of the "primary-acute" form and the serious prognosis.

No further division of the chronic cases in our material has been made concerning either prognosis or course of the disease. We feel that several factors make it difficult to maintain the distinction between the intermittent and the chronic form of the disease, i. e. in view of the fact that serious X-ray changes can be apparent although there are no subjective complaints. A classification into various groups according to the radiological involvement of the colitis is often of little value, as the visible changes within the same group vary during different periods of the disease. Observations in connection with operative cases or at autopsy show that the colitis extends further than X-ray has been able to show (3). The method of double contrast introduced by Welin (26) gives better results but has not been adopted in our series. During the acute phase of the disease, X-ray has often been incomplete or could not be made at all. Therefore we did not want to draw any conclusions concerning the extension of the colitis at the follow-up examination as compared to earlier examinations. Only those cases where total remission has occurred with a complete normalisation of earlier X-ray changes, and where simultaneously sigmoidoscopic findings were normal and no subjective symptoms present, warranted tabulation. Such total remission has occurred in 10 per cent of our material, a percentage which might even be higher since 4 patients without subjective

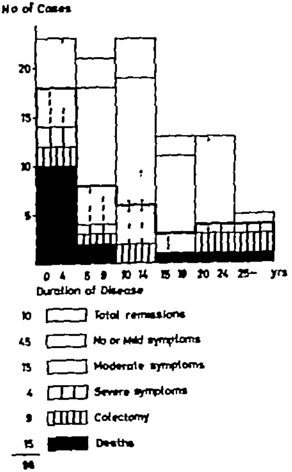


Fig 3 The result of the follow-up examination of 98 cases of ulcerative colitis.

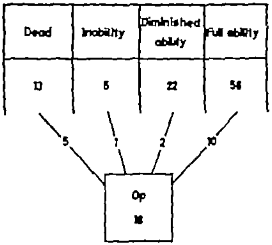


Fig 4 Working capacity at the time of the examination.

colon have been found. 18 received surgical treatment half of them in the acute phase The duration of the disease is over 10 years in 55 % and over 20 years in 18

Discussion

A report has been made covering a material of ulcerative colitis that can truly be called primary material. We use this term because these cases came under our observation right from the start of the disease. The material has been collected during a period of 20 years from one particular district. Little equivalent primary material has been published and indeed perhaps none from a single hospital not specializing in ulcerative colitis. Such material should therefore, even though not extensive, be a very apt basis for an estimate of the nature and the prognosis of the disease. Material in part comparable has been published by Rice-Oxley and Truelove (20) Kellock and White (13) Dick and Grayson (8) and Ihre (1 12)

The frequency of the disease here assessed at 3.5 per 10 000 patients admitted to the hospital is somewhat lower than that usually stated which is 5 0—10.0 per 10 000 This might depend on the fact that the hospital has an unusually high turnover of patients, but it might also reflect the fact that there has not been any large collection of cases of ulcerative colitis. That such a vast collection is possible to a considerable extent is obvious from our material where 25 per cent were readmitted to special clinics. Most of the cases readmitted were in their first 10-year period of our observation.

The proportions between the sexes and the age at the onset of the disease are rather similar to those in other materials published (3) A familial incident of ul-

should appear less often. We wish to stress, however that the risk of malignancy should never be the reason for surgical treatment. The high frequency of malignant degeneration in certain material (5, 17) may be explained by the selective nature of such series. We have not registered the frequency of other complications.

The examination has shown that the prognosis in the individual, serious case is dubious, and worst for those who get the disease acutely and for older patients. This tallies with the findings of other workers (13 20) A much more optimistic picture is seen when looking at the entire unassorted material. We cannot find support for the theory that the very risk of cancer should motivate a higher frequency of colectomies. The operation should mainly be performed upon other indications and there is certainly no shortage of those. We will stress, however that most of those who get ulcerative colitis retain their capacity for work after conservative treatment. Our follow-up examination has further taught us that these cases must be regularly checked and examined as the condition is continuously changeable.

Summary

A true primary material of 98 cases of ulcerative colitis is analysed. There were 5 acute fulminant cases. There were 13 deaths 4 of acute fulminant form, 3 of acute exacerbation of the chronic form, 4 of emaciation, one of cirrhosis of the liver and one of carcinoma. There were 2 cases of carcinoma in the material and those patients had had the disease over 10 years. The indications for colectomy are discussed. It is stressed that colectomy rarely should be indicated on the sole risk of malignant degeneration. The indications are reported on.

The follow-up investigation shows that all 10 patients with total colectomy are in good health. It also shows that about 60 per cent of the material receiving only medical treatment have full working capacity Only 10 per cent have a complete remission. The investigation also points out the necessity for continuous follow-up of cases of ulcerative colitis.

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The patients have received appropriate *medical treatment* After *Salazopyrine* (salicylazosulphapyridine) became available in the market (23 24) this has been the primary remedy used *first and foremost* Later on steroids have been used *mainly in children* and good results have been obtained Steroids have also been used in the acute phases, but here *the effect was not so easy to determine* (7) Due to the intermittent course of the disease it is difficult to evaluate the usefulness of internal therapy and its influence on the supposed course of the disease. *Despite the difficulty of evaluating* the conservative treatment it may be stated that our primary material shows that about 60 per cent have retained their working capacity solely to medical treatment.

During the first 15 years covered by our survey *surgical treatment has*, generally speaking been used only during the active phase of the disease and it is *only during the later period* that our present indications have been formed With the primary-acute fulminant form colectomy should be performed if the medical treatment has not shown an *obvious improvement* within the first week (7) Our failures in this group make it clear that we have been waiting too long before we have operated on those acute resistant fulminant cases. To avoid *deaths* we are now sure it is necessary to perform colectomy sometimes within a week.

With the chronic form an operation should be performed if the disease cripples the patient and *in the case of young* people, if the disease has a retarding effect upon the development of the individual (17) Complications like perforations, abscesses, slowly developing stenoses, *strictures, fistulas polyps or* other changes suspicious of cancer and progressive damage to the liver are also indications for surgery (3) Ileostomy was considered advisable and some *workers still consider it thus*, a view which we do not share. If the patient cannot be saved by medical treatment, ileostomy alone will not help him either unless the affected organ is removed. An *operation should mean total colectomy* possibly in combination with an excision of the rectum. In principle the radical operation should be the right one. Seen from psychological point of view an ileo-*rectal anastomosis must be justified*, if changes in the rectum are non-existent (2) Later series subjected to ileo-rectal anastomosis have shown that the results can be really satisfactory and that milder *changes of the rectum can heal (11)* It is however important that the sigmoid should be totally removed and no part of the colon left

Three cases who mentioned severe *complaints at the time of the follow up* examination have undergone colectomy with good results. *All 10* cases who have undergone colectomy are satisfied and in full working capacity One of them, aged 20 married 10 months after the operation.

In the material *2 cases of carcinoma of the colon* have been found equivalent to 2 per cent of the entire material and 4 per cent of those who have the disease for more than 10 years. Due to the fact that *surgical treatment* nowadays is considered more often than previously this might mean that malignant degeneration

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Fibrinolytic Activity

Inhibition of Plasminogen Activation

By

Johs. Gormsen

In recent years fibrinolytic phenomena have been studied with growing interest. Some authors suggest that an impaired fibrinolytic mechanism may underly the pathogenesis of atherosclerosis. Fibrin deposition is an integral part of all acute inflammatory reactions and the fibrinolytic process is a basic repair mechanism operative throughout the organism (1).

Three types of assay methods for determination of the active proteolytic enzyme plasmin have been used: fibrinolytic assays, proteolytic assays and assays utilizing synthetic substrates such as lysine and arginine esters. Fibrin is the most physiological substrate, and fibrinolytic assays have proven most sensitive but least reproducible. Although the fibrin plate method does constitute a valuable assay method for plasminogen activator and plasmin, it suffers from some disadvantages such as the long incubation period required and its inefficacy in demonstrating reduced activity.

In 1961 Nilsson et al. (5) described a patient who had had since childhood progressive symptoms of massive venous thrombosis. Coagulation studies showed a high content of serum components inhibiting the activation of plasminogen by streptokinase and urokinase. Further they described a method for determination of the inhibitor. The inhibiting component was found in the fraction containing α_2-globulins, it was not dialysable and not adsorbed to $BaSO_4$. It was destroyed by heating at 56° C for 30 minutes. The antiplasmin activity and the trypsin-inhibiting activity of the patient's serum were also increased but could be distinguished clearly from the actual inhibitor by the method described.

The aim of the present work has been to determine the concentration of these serum inhibitors in some patients in whom the fibrinolytic activity might be impaired, to compare the results with those obtained by the plate method, and to examine whether any relation-

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out in 75 normal controls aged 25—70 years with three concentrations of serum 12.5, 25 and 50 %. As controls two blanks were used in which serum was replaced by 0.9 % saline and a solution (0.1 %) of epsilon-aminocaproic acid respectively. Only the results obtained by urokinase (0.05 mg/ml) are given. With the serum concentration of 12.5 % the clot-lysis took 12.5 min. ± 1.5 min. and with the 25 % serum the clot-lysis took 31.4 min. ± 7.2 min. Twenty determinations on the same batch gave clot-lysis times of 12 min. ± 0.4 min. and 24 ½ min. ± 0.6 min. with 12.5 and 25 % respectively. The results in the same person under standard conditions are extremely constant. No correlation with age or sex was demonstrated. No distinct changes after fatty meals were recorded.

Non-complicated atherosclerosis

The values found in 33 patients suffering from atherosclerosis (not diabetic, no acute thrombosis, no necrosis) are shown in fig. 1. The inhibitor-concentration was not high.

Acute heart infarction

The values found at the admission in 23 patients are shown in fig. 2. About one third of the patients had values within the normal range. The 4 patients who had the most retarded clot lysis got a thromboembolic complication, and 3 of them died, the autopsy revealing a mural thrombosis in the heart. One of them died from a ruptured heart. In one third of the patients the inhibitor concentration seemed rather low and the clot-lysis was as quick as in patients suffering from hepatic cirrhosis with latent or manifest haemorrhagic diathesis. In diabetic patients the clot-lysis generally was retarded. During the following weeks

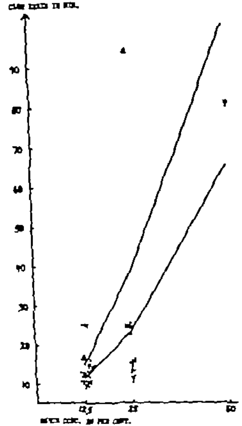


Fig. 3. Fibrinolytic activity measured by Nilsson method in 29 patients with hepatic cirrhosis. Values above 100 min. are not marked out (3 patients, serum concentration 50 %).
Δ = treated with caproic acid.
× = treated with steroids.

the clot-lysis generally became accelerated except in some diabetics and in patients who got thromboembolic or pyogenic complications and in certain others. Treatment with phenylindanedione did not influence the results.

Hepatic cirrhosis

The values found in 29 patients suffering from hepatic cirrhosis are shown in fig. 3. In most cases the diagnosis was verified by biopsy. Otherwise the diag

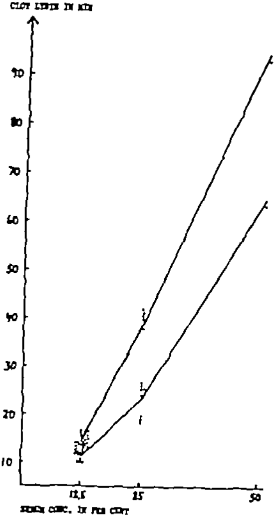


Fig. 1 Fibrinolytic activity measured by Nilsson's method in 33 patients with uncomplicated atherosclerosis. Clot lysis above 100 min. is not marked out (5 patients, serum concentration 50 %) Normal range between the solid lines.

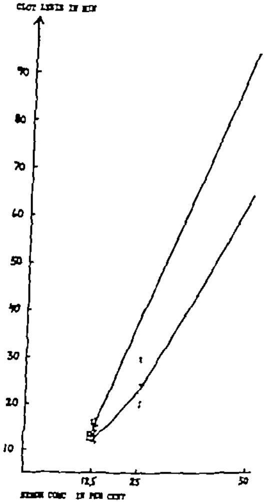


Fig. 2. Fibrinolytic activity measured by Nilsson's method in 23 patients with acute heart infarction. The values given are from the day of admission. Clot lysis above 100 min. is not marked out (10 patients, serum concentration 50 %) Normal range between the solid lines.

ship between fibrinolytic activity and heparin resistance in vitro could be demonstrated

Methods

Serum inhibitors of plasminogen activation. Nilsson's technique (6) was followed in all details and identical preparations of fibrinogen, thrombin, urokinase, streptokinase, plasminogen, buffers and so on were used.

The fibrin plate method assay according to Astrup and Mullertz (2) was carried out by O Storm.

Fibrinogen. The concentration was estimated spectrophotometrically

Heparin-tolerance test was carried out according to the principles earlier described (3)

Results

Normal controls

Determination of serum inhibitors according to Nilsson et al (5) was carried

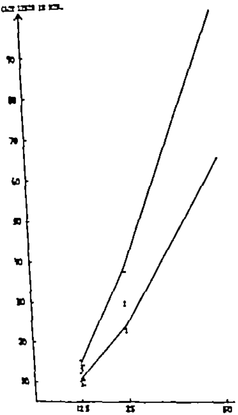


Fig. 5. Fibrinolytic activity measured by Nilsson's method in 19 patients suffering from malignant diseases. Values above 100 min. are not marked out (10 patients, serum concentration 50 %).

prostatic cancer and in one with pulmonary cancer the clot lysis was very quick. These patients did not bleed.

No relationship between the values found and the presence of metastases was demonstrated.

Some other diseases with high inhibitor content

A slight retarded clot-lysis was found in patients with diabetes (14) even before vascular complications were demonstrated, in some patients with acute

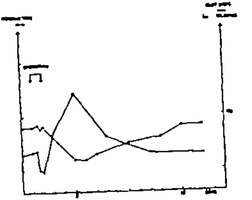


Fig. 6. Inhibitor concentration and heparin resistance after an operation. The concentration of the serum inhibitor and the heparin resistance increased postoperatively.
— = clot-lysis.
x—x = heparin tolerance test.

venous or arterial thrombotic complications (13), with pulmonary infarction (4), with papillitis necroticans in the kidneys, the retardation being severe (3), with gangrene (4), and moreover in patients with acute cholecystitis (3), cystopyelitis (2), arteritis (1) and in patients treated with steroids for temporal arteritis (2) or pancytopenia (2).

Fifty surgical patients were investigated. About 30 % and especially patients undergoing extensive surgery exhibited a very retarded clot-lysis during the first 5(—10) days after the operation (fig. 6).

Some other diseases with low inhibitor concentration

A low concentration of the actual inhibitor was found in 4 patients with thrombocytopenia (less than 5,000 platelets/mm³), in 2 patients with acute myeloblastic leukaemia and in 3 patients who bled copiously after prostatectomy for benign prostatic hypertrophy (2) and one after thyroidectomy.

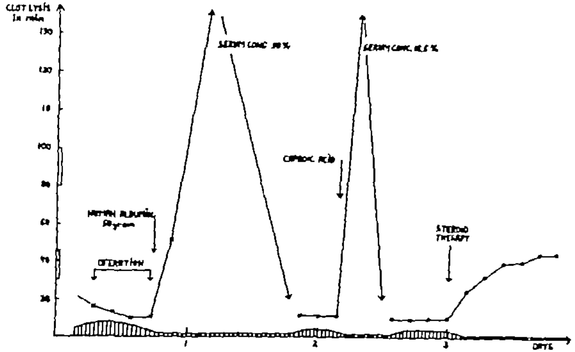


Fig 4 Fibrinolytic activity measured by Nilsson's method in patient with cirrhosis, subjected to a porta-caval shunt operation Lysis-time before operation rather short. It shortened during and after the operation. The patient bled heavily Treatment with ε-aminocaproic acid (6 g intravenously) or human albumin increased the inhibitor concentration and stopped the bleeding temporarily

nosis was based on the usual biochemical tests including a pathological bromsul phalein retention. In most patients the clot lysis was enhanced Four patients under long term treatment with steroids repeatedly had normal values, and in 3 cases treated with ε-aminocaproic acid perorally the inhibitor concentration was high. Only in one untreated patient was the clot lysis retarded She died and at the autopsy a portal thrombosis was found.

In fig 4 the values are shown for a patient subjected to a porta-caval shunt operation. The clot lysed quickly before the operation and during and immediately after the operation the activity was enhanced The patient bled heavily Treatment with human albumin or ε-aminocaproic acid markedly increased the inhibitor concentration in the serum and the bleeding temporarily stopped. Steroids had a similar but smaller effect.

In four patients with acute infectious hepatitis, in seven with obstructive jaundice in 2 with cholangitis and in 4 with malignant diseases in the liver a normal or retarded clot lysis was found.

Malignant neoplasms

The values found in 19 patients suffering from different types of malignant diseases are shown in fig 5 It is well known that pancreatic cancer is often complicated with phlebothrombosis whereas prostatic cancer may be complicated with a haemorrhagic diathesis.

In 3 patients with pancreatic cancer (no jaundice) and in one with rectal cancer the clot lysis was very retarded. No thromboembolic complication was demonstrated In two patients with

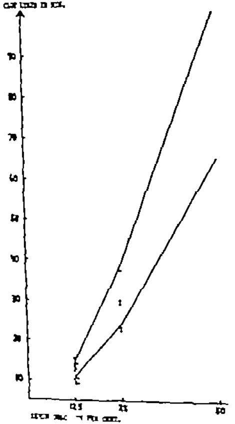


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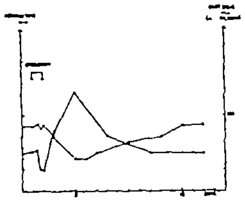


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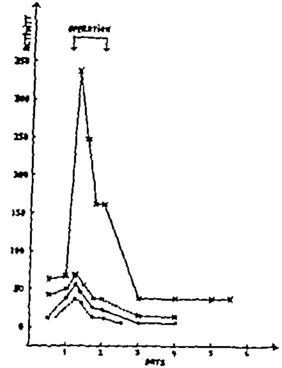


Fig 7 Fibrinolytic activity measured by the plate method in the patient illustrated by fig 4
- – – – = heated fibrin plates.
- x– – –x = unheated fibrin plates.
- x—x = euglobulin fraction.
- •—• = untreated plasma.

Relationship to the fibrin plate method

Nine of 38 investigated patients had increased activator activity on the fibrin plates, especially surgical cases and patients with a cirrhotic liver These nine appeared to have a low inhibitor concentration by Nilsson's method. A further eleven patients had an enhanced clot lysis but no increased activity on the plates. Possibly some activity had been lost as the plasma samples had been kept frozen some days before testing Fig 7 shows the fibrinolytic activity estimated by the plate method in plasma from the operated patient having a porta-caval shunt shown in fig 4

Relationship to the heparin resistance

In many conditions where an increased inhibitor concentration is found especially postoperatively and in acute thrombotic cases, an increase in heparin resistance is found too (fig 6) In other cases an enhanced fibrinolytic activity and a decreased heparin resistance are seen as in patients with cirrhotic liver But the fact that a distinct discrepancy is sometimes found indicates that no direct relationship exists between fibrinolytic activity and heparin resistance.

Maybe, increased heparin resistance in vitro has no correlation to blood coagulation (4)

Relationship to some proteins

In Nilsson's method the concentration of fibrinogen in the patient's plasma is left out of consideration. The fibrinogen solution added before testing contains 96 1 clottable protein. The fibrinogen content in most plasma samples with an enhanced fibrinolytic activity was normal or subnormal, but an elevated concentration was found in some. No distinct correlation was seen between inhibitor concentration and α_1-globulins as demonstrated by paper electrophoresis.

Discussion

Many problems concerning the fibrinolytic phenomena are still unelucidated Our knowledge about the activation of plasminogen and the relationship between the proactivator and plasminogen is very superficial. The character of the different inhibitors and their mode of action is fundamentally unknown It is then not possible definitely to evaluate the results given in this report. The method under investigation has proven to be a very reliable one and without too many technical problems and is carried through within 3 hours.

In some patients a fascinating correlation between the concentration of the inhibitory component and the clinical picture from a haemostatic point of view was obvious, especially postoperatively. In these patients the heparin resistance generally accorded with the content of inhibitors again from a haemostatic point of view. It might be surprising that no increase in the inhibitor concentration was found in patients with atherosclerosis. But this finding does not permit any consequent conclusion about fibrinolytic activity and atherosclerosis. The different values found in patients with acute heart infarction might be of importance from a prognostic and a therapeutic point of view.

In 7 patients with abnormal bleeding tendency and a quick clot-lysis the bleeding stopped promptly after treatment with aminocaproic acid. Similar results were obtained by Nilsson et al. (6).

Summary

A component in serum inhibiting the activation of plasminogen with urokinase has been estimated in 75 normal controls and in 211 patients by the method of Nilsson et al. In some acute thrombotic cases and postoperatively especially after extensive surgery the inhibitor content was high. In atherosclerosis no increase in the concentration was found. In acute heart infarction the concentration varied between distinct increase and decrease. In most cases of hepatic cirrhosis the clot-lysis was quick. In patients with cellular destruction the clot lysis most often was retarded. When a high activator content was found by the fibrin plate method the concentration of the inhibitory component was estimated as low. In many patients a distinct relation between inhibitor concentration and heparin resistance was found. But this relationship was not precise. Increased heparin resistance in vitro might depend on proteins without any known clotting activity.

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Studies in Neurocirculatory Asthenia (Da Costa s Syndrome)

I. Variations with Regard to Symptoms and some Pathophysiological Signs

By

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In neurocirculatory asthenia (NCA) (2, 9 10 11 12, 14 17 35 37 38, 42 63, 65, 67 70 72, 74 75) the diagnosis is suggested from the case history and confirmed by clinical examination. This has made many authors pay little attention to findings in the ECG and the work test. In view of the common occurrence of organic and functional heart disease in the same patient a thorough knowledge of the changes caused from NCA per se, is of outstanding importance. In this paper a report is given of 130 cases of NCA, having the typical clinical picture Special attention is given to abnormal findings in the ECG at rest, in the standing position and after work, and to the physical working capacity (PWC)

The following definition of NCA has been used A condition characterized by the experiencing of abnormal sensations from the heart and the peripheral circulation and by breathing of a characteristic type usually associated with anxiety and tiredness, which cannot be adequately explained by organic heart disease or other organic disease.

Material and methods

The material consists of 130 cases, 44 men and 86 women, 28 of whom have been described earlier (23, 24, 25, 26, 27 28) The range of the age is 17—56 years, with a mean of 35 years. The patients have been treated in the medical clinic from 1940 to 1958 and in the outpatient department from 1955 to 1958 and most of the patients have been seen personally by the author Only patients on whom the work test was performed are considered. The material is thus selected, and cases presenting diagnostic or therapeutic

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Following papers are to be published in this series

II. Statistical analysis of the associations between symptoms and signs in the work test and the correlation between these factors and hemodilution and bodily constitution.

III. On the etiology and the pathogenesis of signs in the work test and orthostatic test.

IV Course during common treatment and physical training and relations between symptoms and signs.

Table I. The frequency of neurocirculatory asthenia among hospitalized patients compared with organic heart diseases over a period of one year

Diagnosis	No. of pts ♂	No. of pts ♀	Total
VOC	25	54	79
Endomyopericarditis	19	21	40
Cardiosclerosis, Cardiac infarction	123	196	319
Arrhythmia	15	16	31
Sympathicotonic orthostatism	3	4	7
Neurocirculatory asthenia	12	18	30
Total	197	309	506
% of NCA in relation to organic heart diseases	6.1	5.8	5.9

Comprises heart disease in essential hypertension. Only monosymptomatic arrhythmias or arrhythmias of dominating clinical importance are recorded.

Table II. Review of the most common symptoms in NCA

Somatic	Precordial pain Palpitations Breathlessness Breathing difficulties Dizziness Cold hands and feet Sweating Numbness Tiredness
Psychic	Sensitivity Anxiety Phobia

problems are probably over-represented. Patients from earlier periods have come for follow-up examination including work test.

The *work test* was performed on a bicycle-ergometer according to Sjöstrand (54, 55, 56) and Wahlund (62). The *ECG* was recorded with the patient at rest in the lying position after 8 min. in the standing position, immediately after work in the lying position, then 4 min. later (for further description see (23)). The PWC is regarded as normal when it amounts to 900 kpm/min. in men and 500 kpm/min. in women (23). PWC normally varies with body parameters. When the result of the work test seems equivocal, as in particularly large or small patients, the PWC has been evaluated in relation to the total amount of haemoglobin (53), which gives a high degree of accuracy (23). The PWC is regarded as being low when the estimated PWC does not amount to the value predicted according to the total amount of haemoglobin, minus once the standard error of estimate. (From this definition about 17 of a normal population is expected to have low PWC.) During the earlier period of this investigation the method for investigation of total haemoglobin was not available. Corrections are then instead made on the basis of the amount of haemoglobin predicted from body weight. Thus in men below 60 kg of weight a working capacity of 750 kpm and in women below 55 kg a working capacity of 400 kpm are regarded as being normal, similarly in men over 80 kg a working capacity of at least 1 000 kpm and in women over 75 kg at least 550 kpm.

In the standing position the pulse rate is regarded as high when it exceeds the value in the lying position by at least 25 beats or when the rate is 100/min. or more. *X-ray* of the chest was routinely performed in the erect position, and heart volume calculated according to Jonsell (32 b).

Statistical methods

The differences are calculated according to the χ^2 method with correction for continuity. The following borderlines for significance are used:

Probably significant	$0.01 < P < 0.05$
Significant	$0.001 < P < 0.01$
Highly significant	$P < 0.001$

Frequency of neurocirculatory asthenia in hospitalized heart cases

About one third to one half of all patients with cardiac symptoms have NCA (8, 4, 64). In order to determine its frequency compared with organic heart disease in hospitalized patients, the number of admissions during one year (1955) was calculated (table I).

Table III. Frequency of some common symptoms in all material and in different groups of NCA with regard to signs at orthostatic test and work test (see text)

Group of NCA with regard to signs	Total no. of pts	Precordial pain		Palpitations		Breathlessness		Breathing abnormality		Peripheral symptoms		Anxiety	
		No. of pts	%	No. of pts	%	No. of pts	%	No. of pts	%	No. of pts	%	No. of pts	%
No abnormal findings	27	24	88.8	14	51.8	10	37.0	5	18.5	18	66.7	23	85.2
Sympathicotonic orthostatism	16	15	93.8	7	43.6	4	25.0	5	31.3	6	37.5	8	50.0
Abnormal ECG	36	27	75.0	27	75.0	21	58.3	14	38.9	29	80.6	32	88.9
Low PWC	51	39	76.5	42	82.3	27	53.0	19	37.3	45	88.2	47	92.1
Total	130	105	80.8	90	69.2	62	47.7	43	33.1	98	75.3	110	84.6

Include dizziness, cold hands and feet, and headache.

Table IV. Association of the most common symptoms in the material. The total number of patients suffering from symptom is given within frame. The frequency of associated symptoms is given in number and percentage

	Precordial pain		Palpitations		Breathlessness		Breathing abnormality		Peripheral symptoms	
	No	%	No.	%	No.	%	No.		No.	%
Precordial pain	105	100	67	64	52	50	35	33	81	77
Palpitations	67	75	90	100	47	52	30	33	78	87
Breathlessness	52	84	47	76	62	100	19	31	50	81
Breathing abnormality	35	83	30	71	19	45	43	100	38	89
Peripheral symptoms	81	83	78	81	50	52	38	39	98	100

Include dizziness, cold hands and feet, and headache.

The hospitalization of patients with NCA was at that time decided upon for practical reasons only. During that year 7 patients, corresponding to 1.4 of all heart cases, presented arterial orthostatic anaemia (4) or sympathicotonic orthostatism (48) but had no heart complaints, and thus, no NCA. They were investigated because of fainting, prostration, etc. The frequency of NCA (5,9) illustrates that diagnostic and therapeutic problems, leading to hospitalization, occur frequently during the course of NCA.

Review of symptoms

The many and varied symptoms in NCA have been repeatedly and consistently described (1 2, 9 10, 12, 14 30 37 38 39 63 64 67 70 73 74 75). More than the symptom itself its type, time of occurrence and duration are characteristic for NCA. There are somatic as well as psychical complaints, the tormenting tiredness being intermediate. The most important symptoms are given

Table V Review of the most common signs in NCA

Pulse and blood pressure fluctuations
Dermographia
Sympathicotonic orthostatism
Depression of the S-T segments and/or flattening or inversion of the T-waves
Low physical working capacity
Sighing respiration
Abnormal breath holding test
Brisk tendon reflexes

in table II Besides these, many patients have symptoms arising from other organs such as gastro-intestinal disturbances. These may occur at the same time as or alternate with circulatory symptoms.

The frequency and association of some of the most common symptoms in this study are given in tables III and IV Some patients suffered from only one symptom such as precordial pain or palpitation and they are referred to here as "partial NCA. Most patients, however had several symptoms, somatic and psychic. They are divided into two groups, NCA somatic and NCA psychic, according to whether the clinical picture was dominated by somatic or psychic complaints. Of course, the borderline between these groups is somewhat subjective. However when studying the pathogenesis of NCA it is of interest to know whether there are any differences between the findings in these two groups.

Review of signs

The most common and easily observed signs which reflect different functional disturbances are reported in table V In this material special attention is given to the findings in the work test and orthostatic test, which are reported in table VI and VII where also the frequency in males and females and the coincidence between different findings are given *ECG changes* with depression of the S—T segment and/or lowering or inversion of the T waves are located diffusely over the left precordium and sometimes also over the right precordium Sometimes they appear *at rest*, and may be seen at ordinary heart rates though they are more common with tachycardia and *after work*. The changes vary in degree and to a certain extent also in pattern and are of a kind repeatedly observed during increased sympathetic tone and under the influence of adrenalin and in anxious patients (15 41 44 45 47 55) as well as in NCA (see Discussion) They are commonly referred to as sympathicotonic For thorough description and definition of these changes the reader is referred to Holmgren et al. (25) An illustration of the ECG changes is given in fig 1

ECG changes of the same type often occur *in the standing position* (76) with or without tachycardia in people without heart disease, who present arterial orthostatic anaemia (4) or sympathicotonic orthostatism (48) They have no symptoms at all or complain of dizziness, tendency to fainting and headache during prolonged standing When palpitations or other heart symptoms are added, the clinical picture develops into NCA. People with NCA often present orthostatic complaints and sympathicotonic orthostatism (tables VI—VII)

The most important disturbance seems to be *low physical working capacity* (PWC) observed in 51 cases which were of course, thoroughly investigated The size and configuration of the heart on X ray were normal. ECG changes indicating localized myocardial damage

Table VI. Abnormal findings at work test and orthostatic test. ECG changes and marked orthostatic pulse reaction in all material, in males and females, and in groups with normal and low physical work capacity (see text)

Sex and group	Total no. of pts	Sympathicotonic S-T and T changes						Marked orthostatic pulse reaction	
		At rest		After work		Standing			
		No.	%	No.	%	No.	%	No.	%
Males	44	1	2.3	9	20.5	13	29.5	24	54.5
Females	86	25	29.1	42	48.8	54	62.8	53	61.6
Group I-III (normal PWC)	79	11	13.9	25	31.6	34	43.0	34	43.0
Group IV (low PWC)	51	15	29.4	26	51.0	33	64.7	43	84.3
Total	130	26	20.0	51	39.0	67	52.0	77	59.0

Table VII. Association of abnormal findings at work test and orthostatic test. The figures within frame represent total number of changes, the other figures give the number and percentage of associated findings (see text)

	Marked orthostatic pulse reaction		Marked orthostatic ECG changes		ECG changes after work		Low PWC		ECG changes at rest	
	No.	%	No.	%	No.	%	No.	%	No.	%
Marked orthostatic pulse reaction	77	100.0	54	70.1	36	46.8	43	55.8	20	26.0
Marked orthostatic ECG changes	54	80.6	67	100.0	44	65.7	33	49.3	24	35.8
ECG changes after work	36	70.6	44	86.3	51	100.0	26	51.0	24	47.1
Low PWC	43	84.3	33	67.4	26	51.0	51	100.0	15	29.5
ECG changes at rest	20	76.9	24	92.3	24	92.3	15	57.7	26	100.0

were observed in exceptional cases, which were further investigated by cardiac catheterization (for methods see Holmgren et al. (23)). This investigation showed a normally functioning heart as judged from stroke volume and intracardiac pressures at rest as well as during work, so the lowering of the working capacity could not be caused by organic heart disease.

Organic heart disease and arrhythmia are far from uncommon in NCA, and often they have been of ætiological importance (6 12 14 17 19 55 63 70 73). In mild organic disease the true nature of the complaints is often obvious, and 11 such cases are included in this material. These cases are specially indicated in the histogram of the material arranged in different groups (fig. 2) and two of them are reported later on (p. 672). In more advanced heart disease also it is often possible to differentiate between organic and NCA symptoms, though

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The most important disturbance seems to be *low physical working capacity* (PWC) observed in 51 cases which were of course, thoroughly investigated The size and configuration of the heart on X ray were normal ECG changes indicating localized myocardial damage

Table VIII. Breathing abnormality reflected in high residual volume in spirometric investigation in a NCA patient. Three investigations were made before, and fourth investigation after breathing exercises

	April 30th 1937 (I)	Jan. 8th 1938 (I)
Absolute values		
Inspiratory capacity	1.39	1.57
Tidal volume	0.56	0.73
Expiratory reserve volume	1.02	1.66
Vital capacity	2.97	3.98
Residual volume (RV)	3.09	1.90
Total lung capacity (TLC)	6.06	5.88
Relative values	(%)	(%)
Functional residual capacity/TLC	65	59
RV/TLC	51	32

Almost identical values were recorded in two earlier investigations.

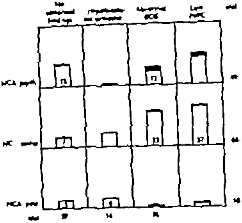


Fig. 2. Distribution of the material.

The patients are divided into groups with regard to symptoms as well as signs on the lines explained in the text. The inked-in parts of the blocks indicate patients with organic heart disease also. The figures indicate total number of patients.

breathing level with involuntary fixation of the thoracic cage, so that the pulmonary capacity could not be utilized to a normal degree.

Classification with regard to signs

With regard to findings at work test and orthostatic test, the following classification of the material is made.

Group I (*No abnormal findings*) comprises cases with no objective findings at all or insignificant aberrations, such as transient tachycardia and blood pressure elevation.

Group II (*Sympathicotonic orthostatism*) has a high pulse rate and/or marked ECG changes after 8 min. in the standing position. No other changes.

Group III (*Abnormal ECG*) includes patients who present ECG changes at rest and/or after work with or without sympathicotonic orthostatism but who have normal PWC.

Group IV (*Low PWC*) comprises all cases with low PWC with or without other signs as mentioned above.

Review of the material

A review of the total material is given in fig. 2 with classification with regard to signs and symptoms. All groups of signs were found in all of the clinical types, just as they may sometimes be found in patients without cardiac symptoms. Absence of abnormal findings was more common in patients with psychic symptoms than in those with somatic symptoms and the difference, 19.0 $\pm$ 7.8 % is probably significant.

In all groups were observed patients with signs of organic heart disease but this was obviously not the cause of the heart symptoms. A report of two of these cases is given below.

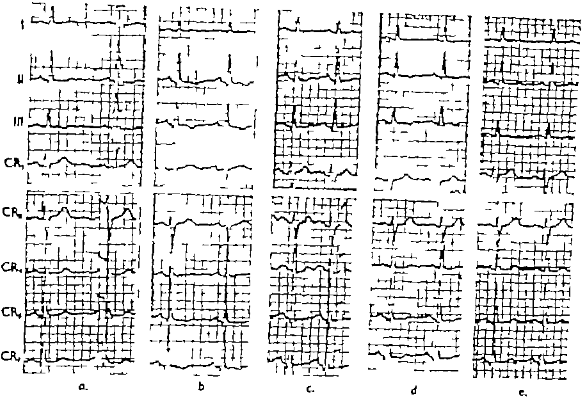


Fig 1 ECG changes in a 37 year-old woman with neurocirculatory asthenia.

a. ECG at rest. Frequency 75. Slight flattening of the T waves in leads II, CR_4 and CR_7.

b. ECG after 8 min. in the standing position. Frequency 95. In spite of the moderate increase in pulse rate the ECG changes are marked with negative T waves in leads II III CR_4 and CR_7, and flattening of the T waves in lead I and CR_4

c. ECG in the lying position immediately after work (200 kpm and 400 kpm during 6 min.) Frequency 135—120 ECG reaction within normal limits with the S—T segments originating below the isoelectric lin

d. ECG 4 min. after work. Frequency 100. Abnormal ECG reaction with depression of the S—T segment in leads CR CR_4 and CR_7, and flattening of the T waves in leads II and CR_1 and inversion of the T waves in leads CR_4 and CR_7.

e. ECG 10 min. after work. Frequency 90. Regression of the ECG changes after work. ECG is of the same type as at rest.

reports of such cases are beyond the scope of this paper

Breathing abnormalities such as sighing respiration, a slight increase in breath holding time after hyperventilation (5, 20 50 63 68) and hyperventilation (7 9 10 11 22 32 42 61 72) are common and well known in NCA. Other types of breathing disturbance may also be observed (52) and a less common type is illustrated by the following case

A 35-year-old male demonstrated during the work test a strange superficial, snapping breathing Three spirometric investigations on different occasions showed almost identical figures with increase of the residual volume (table VIII) X-ray of chest, lungs and spine as well as lung compliance and diffusion capacity were normal. Kymographic breathing studies showed big excursion of the diaphragm but very small movements of the ribs. After breathing exercises the spirometry was normal (table VIII) The cause seems to have been a high resting

Table VIII Breathing abnormality reflected in high residual volume at spirometric investigation in a NCA patient. Three investigations were made before and a fourth investigation after breathing exercises

	April 30th 1957 (l)	Jan. 8th 1958 (l)
Absolute values		
Inspiratory capacity	1.39	1.57
Tidal volume	0.56	0.75
Expiratory reserve volume	1.02	1.66
Vital capacity	2.97	3.98
Residual volume (RV)	3.09	1.90
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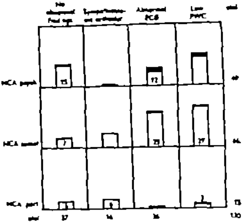


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In all groups were observed patients with signs of organic heart disease but this was obviously not the cause of the heart symptoms. A report of two of these cases is given below

A 35-year-old woman belonged to group I with no signs but with dominating somatic complaints. In 1953 she awoke one night with breathlessness and a feeling of fainting. ECG revealed prolonged P—Q time 0.25 sec. and the patient was informed that she had heart disease. A few months later there was another similar attack. After that she was symptom-free till 1955, when she became tired and irritable, and had precordial pains, palpitations and sweating. During these two years of observation, P—Q time varied between 0.24 and 0.28 sec. at rest with shortening in standing position and during and after work. X-ray of the heart and the PWC was normal. It was thought that the A—V block was probably the sequela of a myocarditis, and that the symptoms in 1955 were due to NCA. This was explained to the patient, whose only complaint two years later was palpitations, which did not worry her.

A 19-year-old man reported as case VA 12 (26) was investigated because of slight palpitations. Throughout the period of observation, nine months, the ECG at rest showed localized elevation of the S—T segment and inversion of the T wave, which was maximum in precordial lead CR. This was probably the sequelae of an asymptomatic perimyocarditis. Besides, in the standing position depressions of the S—T segments and inversion of the T waves over the left precordium of sympathicotonic type appeared. The PWC was low. Right-sided heart catheterization showed normal pressures and normal stroke volume but low a—v oxygen differences at rest as well as during work. This functional disturbance and not the organic disease seemed to be the explanation of the low PWC. After hard physical training the PWC became normal and the sympathicotonic ECG changes in the standing position vanished while the localized ECG changes remained. The patient was symptom free and doing heavy work. This case is reported as "partial NCA" in group IV.

Patients with low PWC had a higher incidence of abnormal ECG findings in the work test and orthostatic test than patients with normal PWC (table VI). The difference is probably significant ($0.01 < P < 0.05$) with regard to ECG changes after work (19.4 % ± 8.8 %) and in the standing position (21.7 % ± 8.8 %) and highly significant with regard to high orthostatic pulse rate (41.3 % ± 7.5 %, $P < 0.001$). All these changes were more common in females than in males, and the difference was highly significant ($P < 0.001$) with regard to ECG changes at rest (26.8 % ± 5.4 %) and in the standing position (33.3 % ± 8.7 %) and significant with regard to ECG changes after work (28.3 % ± 8.2 %) ($0.001 < P < 0.01$). There is no significant difference with regard to low PWC between males and females.

Discussion

Among all symptoms in NCA precordial pain is of special interest. The pathogenesis is obscure, and many hypotheses have been presented (19, 20 a. o.). In this material no correlation was found between precordial pain and ECG changes which, as all other experiences suggest, is against ischaemia being their cause (table III). The associations with the various other symptoms were about equal except that there was little correlation between palpitations and precordial pain (table IV). In groups III and IV with abnormal ECG findings and low PWC, precordial pain and palpitations were about equally common (table III), but in groups I—II with no abnormal findings or only sympathicotonic orthostatism precordial pain was more common than palpitations (the difference being 42.0 % ± 9.0 %). This difference was observed in both males and females, despite palpitations being less common in men than in women.

Besides, palpitations had a significantly lower frequency in groups I—II than in groups III—IV with a difference of 30.9 % ± 8.9 %, but with regard to precordial pain the tendency was the reverse. This seems to indicate, that palpitations are combined with ECG changes and low PWC to a greater degree than is precordial pain. Further statistical analyses are to be given in a subsequent paper (Levander Lindgren and Ek).

Regarding *ECG findings* varying reports are given and for extensive review the reader is referred to Friese and Haid (18). Several authors have observed depression of the S—T segment and/or lowering or inversion of the T waves in patients with NCA without signs of organic heart disease (13 16, 21 29 31 42, 43 58, 58 b, 64 66 71) while other authors emphasize that there are no ECG changes characteristic of NCA (11 37 49 69) or even regard a normal ECG as essential for the diagnosis of NCA (46). In the Framingham study Kannel et al. (33) did not observe a higher incidence of *ECG* changes in people presenting symptoms of NCA, than in healthy controls. Similar observations were made by Friese and Haid (18) but they found on the other hand that the height of the T wave lead 2 was definitely lower in people with NCA than in the controls. The different opinions may be due to different selection of the case material. One can *hardly* expect the same findings in NCA patients during acute disease as in symptom-free people who have reported NCA symptoms when answering a questionnaire. This is especially true because there are marked and sometimes pid ariations of the ECG findings in NCA as reported by Holmgren et al. (26 28) and as will also be described in a subsequent paper (Levander Lindgren, to be published).

This study demonstrates that depression of the S—T segment and lowering or inversion of the T waves at rest occur not infrequently in women during the course of NCA (29 %) but the changes are less common in men (2 %). Marked ECG changes after work are a common finding in women (49 %) but are less often observed in men (21 %).

Marked orthostatic reactions with high pulse rate and/or marked depression of the S—T segment and lowering of the T wave in the standing position are not pathological per se, but the frequencies, 52 % and 59 % respectively were strikingly high in this NCA material. Similar observations were made by Åkesson (77). Linko, studying the orthostatic reaction in soldiers with NCA, also found the pulse increase higher than normal, but the ECG reaction did not differ significantly from that in a control group of healthy men (40). The ECG changes in the erect position in the present material were more common in women 63 % against 30 % in men which was also observed by Friese and Haid (18). The difference with regard to pulse rate is, however insignificant. The influence of sex may explain the difference between Linko and the present material.

Marked pulse and ECG reactions in the standing position were, as a rule associated but either may appear alone (table VII). In patients with low PWC a marked pulse reaction was found in 84.3 % and a marked ECG reaction in 64.7 %. ECG changes after work were more frequent with marked orthostatic ECG reaction (65.7 %) than with marked orthostatic pulse reaction (46.8 %). These findings indicate a

positive correlation between ECG changes in the standing position and after work, and between high pulse rate in the standing position and during work. Statistical analysis will be given in a subsequent paper (Levander Lindgren and Ek)

Attention is seldom paid to the PWC in NCA. Master (46) stated that a normal PWC is a criterion of functional heart disease while Lewis (37) and Cohen and White (9) observed that the PWC was lower in NCA cases than in healthy people, and Stevenson (cit. Wolf) reported a decrease of pulse rate after a standard exercise test in connection with improvement of NCA. In this material low PWC was observed in no less than 39 % and ECG changes and marked orthostatic reactions were more common among these patients than among the patients with a normal PWC.

The same aberrations during the work test, which were often found in patients with NCA, may be observed in patients having no NCA symptoms at all. One of these patients, who was occasionally observed by the author and not reported in this paper later on developed NCA. NCA symptoms and the described objective findings at work test may thus appear independently of each other though they are often associated

Summary

A report is given of 130 cases of neurocirculatory asthenia (NCA) with regard to symptoms, especially the frequency of precordial pain and palpitations, and to findings during the work test and the orthostatic test with recording of ECG It is shown that the signs vary markedly In females sympathicotonic changes of the S—T segment and the T waves were frequently observed after work (49 %) and less frequently at rest (21 %) In males they were less common the incidences being 29 % and 2 % respectively Marked ECG changes in the standing position were also common especially in females, while high pulse rate was observed with about the same frequency in both sexes. Low physical working capacity was observed in 39 % and these patients had a higher frequency of ECG changes and of marked orthostatic reactions than other patients.

A positive correlation was evident between ECG changes after work and in the standing position on the one hand, and a high pulse rate in the standing position and during work on the other Palpitations were linked with ECG changes and low PWC to a greater extent than was precordial pain. Further statistical analysis of these correlations will be given in a subsequent paper

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Studies in Neurocirculatory Asthenia (Da Costa's Syndrome)

II. Statistical Analysis of the Associations between Symptoms and Signs in the Work Test and th Orthostatic Test, and th Correlation between these Factors and Immobilization and Bodily Constitution

By

Maj Levander Lindgren and Stig Ek

Patients with neurocirculatory asthenia (NCA) present a high degree of variability with regard to both symptoms and signs in the work test and the orthostatic test, as was reported in a preceding paper in this series (7). To find out the relations within the disease of the most important symptoms and signs as well as the association between them and immobilization and bodily constitution statistical analysis has been performed.

Association coefficients between symptoms and signs

The association coefficients, V (Kendall) between the most important symptoms and signs have been calculated from the respective four-field tables, giving the number of patients having the four possible combinations of presence and lack of the two respective variables. The association coefficient varies like the correlation coefficient between + 1 for absolute association, through 0 for complete lack of association to — 1 for absolute negative association. The value for each coefficient is given in table I. For criteria determining the abnormal findings see preceding paper (7).

One of the variables introduced here is immobilization, this signifying that the patients do not perform any work but are not bedridden (for further definition see Levander Lindgren (6)). This factor is of special interest with regard to its relation to low physical working capacity (PWC) and marked orthostatic pulse reactions (OPR). Values for all associations are calculated. The significance is tested and given in table I.

Among *symptoms* (left upper quadrant in table I) palpitations and marked anxiety are positively correlated to each other and they show [?] significant negative correlation to precordial pain. All symptoms with the exception of precordial pain show a significant positive correlation to immobilization.

The correlations between different *signs* are given in the right lower quadrant in table I. There is [?] highly significant positive correlation between ECG changes at rest, after work and in the standing position on one hand, and between high orthostatic pulse rate (OPR) and low PWC on the other. Between the other signs the correlations are slightly significant.

The correlations between *symptoms and signs* are indicated in the right upper and left lower quadrants in table I. There are slightly significant correlations between immobilization [?] and low PWC and between palpitations

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The correlations between *symptoms and signs* are indicated in the right upper and left lower quadrants in table I. There are slightly significant correlations between immobilization and low PWC and between palpitations

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Positive or negative correlations and the significance according to χ^2 test are indicated. The significances are ... definitions of the factors and are thus of less interest

8	9	10	11	12	13	14	15
+0.20*	+0.17	+0.19	+0.03	−0.00	+0.16	+0.16	+0.18
−0.21	(+0.13)	−0.10	+0.05	+0.03	−0.05	−0.09	−0.09
+0.28	(+0.39***)	+0.17	+0.17	+0.25	+0.06	+0.07	+0.22
−0.03	(+0.25**)	+0.09	+0.06	+0.10	+0.13	−0.01	+0.10
—	(+0.28)	−0.21	+0.13	+0.08	−0.02	−0.05	+0.04
(+0.38**)	—	+0.06	+0.18	+0.19	+0.03	+0.01	+0.14
−0.21	+0.06	—	(+0.26*)	(+0.41)	(+0.51)	(+0.61)	(+0.40*)
+0.13	+0.18*	(+0.26**)	—	+0.56**	+0.42**	+0.18	+0.19
+0.08	+0.19*	(+0.40)	+0.55*	—	+0.56***	+0.21	+0.21
−0.02	+0.03	(−0.31 **)	+0.42	+0.56*	—	+0.46	+0.21
−0.05	+0.01	(+0.61)	+0.18*	+0.21	+0.46**		+0.40**
+0.04	+0.14	(+0.40 **)	+0.19*	+0.21	+0.21	+0.40	—

Table II. Association coefficients between the most characteristic variables in NCA observed by factor analysis, and immobilization. For definitions see text and table I

		4	9a	12	14	15
Immobilization	4	—	+0.29 ( )	−0.00	+0.16	+0.18
At least 2 of 3 main symptoms	9a	+0.29** ( )	—	−0.15	−0.02	+0.12
ECG changes after work	12	−0.00	+0.15	—	+0.21	+0.21
Marked OPR	14	0.16	−0.02	+0.21	—	+0.40**
Low PWC	15	−0.18*	+0.12	−0.21	+0.40*	—

3. One objective component, which is most purely represented by high OPR and is well related to low PWC (= high pulse rate during work. This component is therefore called High pulse rate component. The nearer symptom or sign is located to corner in the triangle the more of the corresponding component does it represent. The 3 components are thus best represented by 8 anxiety 14, marked orthostatic pulse reaction, and 12, ECG changes after work. Marked orthostatic ECG changes, 13, thus contains mostly the ECG component but also the high pulse rate component although not the subjective symptom component. The figure shows that the symptoms and signs with the exception of precordial pain, 5, contain only one or two of the components, i. e. they are situated either on corner or on side in the triangle. Precordial pain, the most common symptom in this material, is either uncorrelated or negatively correlated to other symptoms and signs.

Associations between the most characteristic variables and immobilization

T characterize the case material system has been chosen with the variables 12 ECG changes t work, 14 high orthostatic pulse rate and new variable, 9 t least 2 of the symptoms palpitations, breathlessness and marked anxiety. The correlations between them and 4 immobilization and 15 low PWC are given in table II with the degree of significance calculated by the same method as in table I. The variables 9 a, 12 and 14 are chosen in such manner that they represent the 3 different components of the disease,

Table I. Association coefficients between the most important symptoms and signs. For definition see text. calculated from association coefficients with 3 decimals. Values within brackets are partly determined by the

		4	5	6	7
Immobilization	4	—	−0.04	+0.23	+0.20
Precordial pain	5	−0.04	—	−0.24	+0.08
Palpitations	6	+0.23	−0.24*	—	+0.16
Breathlessness	7	+0.20	+0.08	+0.16	—
Marked anxiety	8	+0.20	−0.21	+0.20	−0.03
More than one symptom.	9	+0.17	(+0.13)	(+0.40)	(+0.25)
Abnormal findings at work test and/ or orthostatic test	10	+0.10	−0.10	+0.17	+0.09
ECG changes at rest	11	+0.03	+0.03	+0.17	+0.06
ECG changes after work	12	−0.00	+0.03	+0.25	+0.10
Marked orthostatic ECG changes.	13	+0.16	−0.03	+0.06	+0.13
Marked orthostatic pulse reaction.	14	+0.16	−0.09	+0.07	−0.01
Low PWC	15	+0.18	−0.09	+0.22	+0.10

= 0.01 < P < 0.05, = 0.001 < P < 0.01 = P < 0.001

and ECG changes after work and low PWC. More than one symptom gives a slightly significant positive correlation to ECG changes at rest and after work. Marked anxiety has a slightly significant negative correlation to "abnormal findings". It must be stressed that there is no significant correlation between precordial pain and ECG changes at rest and after work.

Factor analysis

Table I gives the relations between the various symptoms and signs taken two at a time. A great variability of the association coefficients has been found. This indicates that several components may be represented in this case material. The structure of the material with regard to symptoms and signs can be further clarified through factor analysis, which takes into consideration the correlation table as a whole and seeks a simple underlying structure, if such a structure exists.

Such an analysis has been performed on the values in table I. The method used is the "centroid method" (10, p. 149) which Thurstone has used on tables of correlation coefficients from psychological investigations.

By the factor analysis it has been found that 3 components represent most of the associations in table I. A simple structure in the sense used by e. g. Thurstone (page 125, 319) has

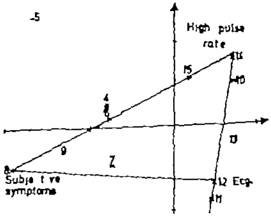


Fig. 1. Factorial structure with regard to some symptoms and signs. The figures correspond to those given in table I. The negative figure −5 indicates absence of precordial pain (= 5)

been found in the system. This structure is indicated in fig. 1 according to the method of extended vectors (10, p. 225). The corners of the triangle indicate the 3 possible "pure" components in the condition NCA. These components are

1. Subjective symptoms with marked anxiety, palpitations and breathlessness. This component is related to immobilization.

2. One objective component, which manifests itself as "sympathicotonic" ECG changes at rest and after work, and may be called ECG component

...itive or negative correlations and the significance according to χ^2 test are indicated. The significances are ...ndings of the factors and are thus of less interest

	9	10	11	12	13	14	15
+0.20*	+0.17	−0.10	+0.03	−0.00	+0.16	+0.16	+0.18
−0.21	(+0.13)	−0.10	+0.05	+0.03	−0.05	−0.09	−0.09
+0.26*	(+0.39*)	+0.17	+0.17	+0.25	+0.06	+0.07	+0.22
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+0.28*)	–	+0.06	+0.18	+0.19*	+0.03	+0.01	+0.14
−0.21	+0.06	–	(+0.26**)	(+0.41***)	(+0.51)	(+0.61)	(+0.40***)
+0.13	+0.18	(+0.26*)	–	+0.36*	+0.42	+0.18	+0.19
−0.08	+0.19*	(+0.40***)	+0.55**	–	+0.56*	+0.21	+0.21
−0.02	+0.03	(+0.51**)	+0.42*	+0.56***	–	+0.46	+0.21
−0.05	+0.01	(+0.61 **)	+0.18	+0.21	+0.46	–	+0.40**
+0.04	+0.14	(+0.40 **)	+0.19	+0.21	+0.21	+0.40**	–

Table II. Association coefficient between the most characteristic variables in NCA, observed by factor analysis and immobilization. For definition see text and table I

		4	9a	12	14	15
Immobilization	4	–	+0.29**( )	0.00	−0.16	−0.18
A least 2 of 3 main symptoms	9a	+0.29* ( )	–	−0.15	0.02	+0.12
ECG changes after work	12	−0.00	+0.15	–	+0.21	+0.21
Marked OPR	14	−0.16	−0.02	−0.21		−0.40**
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To characterize the case material a system has been chosen with the variables 12 ECG changes at work, 14 high orthostatic pulse rate and new variable, 9 a at least 2 of the symptoms palpitations, breathlessness and marked anxiety. The correlations between them and 4 immobilization and 15 low PWC are given in table II with the degree of significance calculated by the same method as in table I. The variables 9 a, 12 and 14 are chosen in such manner that they represent the 3 different components of the disease

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More than one symptom.	9	+0 17	(+0 13)	(+0 40)	(+0.25)
Abnormal findings at work test and/ or orthostatic test	10	+0.10	−0.10	+0.17	+0.09
ECG changes at rest	11	+0.03	+0.03	+0 17	+0.06
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− 0.01 < P < 0 05 − 0.001 < P < 0.01 − P < 0 001

and ECG changes after work and low PWC. More than one symptom gives a slightly significant positive correlation to ECG changes at rest and after work. Marked anxiety has a slightly significant negati e correlation to "abnormal findings" It must be stressed that there is no significant correlation between precordial pain and ECG changes at rest and after work.

Factor analysis

Table I gives the relations between the various symptoms and signs taken two at a time. A great variability of the association coefficients has been found. This indicates that several components may be represented in this case material. The structure of the material with regard to symptoms and signs can be further clarified through factor analysis, which takes into consideration the correlation table as a whole and seeks a simple underlying structure, if such a structure exists.

Such an analysis has been performed on the values in table I The method used is the centroid method" (10, p. 149) which Thurstone has used on tables of correlation coefficients from psychological investigations.

By the factor analysis it has been found that 3 components represent most of the associations in table I. A simple structure in the sense used by e g Thurstone (page 125, 319) has

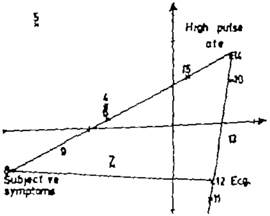


Fig 1 Factorial structure with regard t some symptoms and signs. The figures correspond to those given in table I. The negative figure −5 indicates absence of precordial pain (−5)

been found in the system. This structure is indicated in fig 1 according to the method of extended vectors (10, p. 225 The corners of the triangle indicate the 3 possibl pure" components in the condition VCA. These components are

1 Subjective symptoms with marked anxiety palpitations and breathlessness This component is related to immobilization

2 One objective component wh ch manifests itself as "sympathicotoni ECG changes at rest and after work, and may be called ECG component"

Table IV. Association coefficients between sex, age and body index and the associations to the main components in the condition NCA, observed by factor analysis (see also text)

		1	2	3
Males	1	—	−0.10	−0.02
Older patients	2	−0.10	—	+0.27
High body index	3	−0.02	+0.27	—
Immobilization	4	−0.12	+0.14	−0.00
At least 2 or 3 of main symptoms	9	−0.07	+0.14	+0.04
ECG changes after work	12	−0.31**	−0.13	+0.02
Marked OPR	14	−0.07	−0.12	−0.21
Low PWC	15	+0.09	−0.01	−0.00

Height and weight crudely reflect the habitus, and height and total blood volume probably gives more valuable information (2) but this parameter was not available in all cases in this series. Åkesson (11) observed correlation between the body index weight/height³ (B) and lowering of the T wave in limb lead III in the standing position. He also found correlation between lowering of the T wave and high pulse rate in the standing position.

I this case material the average height and weight were in men 178.8 cm and 72.6 kg and in women 163.7 cm and 57.7 kg. In small normal material from the same population as the NCA patients (2) the average height and weight were in men 177.3 cm and 70.0 kg and in women 164.3 cm and 57.0 kg. The dimensional factors do not differ significantly from those in this control case material, but statistical analysis with regard to body index is further performed below.

Body index weight in kg/height³ in m is calculated and recorded for men and women in fig. . The index is also calculated for different subgroups used in this series (7). The difference between these groups is not significant.

The average body index is 12.3 for males with marked OPR and 13.2 with normal OPR, and for females 12.8 with marked OPR and 13.7 with normal OPR. The difference is significant for females ($0.01 < P < 0.02$) but not for males. The correlation between body index and OPR has also been tested with analysis of variance by using the separate values for each patient. This gives a significant difference ($0.001 < P < 0.01$) between patients with marked and normal OPR.

Another way of analyzing the associations between body index and other components in the condition NCA is to divide the case material into two equal parts, one with relatively high and one with relatively low body index. The division is here made at index 13.0 and there are 20 males and 40 females in the high index group, 24 males and 44 females in the low index group. In table IV the associations between high body index, 3, and marked OPR and other factors here studied are given. As above one finds a significant negative correlation between high body index and marked OPR but no significant correlation between high body index and low PWC.

In table IV are further given the associations with regard to sex and age. Males are indicated with factor 1 positive, females with factor 1 negative. Factor 2 positive indicates older patients (≧ 35 yrs), factor 2 negative younger patients (< 35 yrs). With regard to body index there is observed a significant positive correlation between older patients and high body index, corresponding to the well-known fact that older persons are fatter than younger. This correlation does, however, not bring about a corresponding negative correlation between older patients and marked OPR of significant degree. There is further to be noticed a highly significant negative correlation between males and ECG changes at work, as observed earlier (6).

Discussion

Factor analysis has shown that there are three main components constituting the

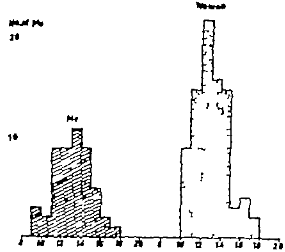


Fig 2 Distribution of the body index weight in kg/height in m in the whole material. The number of patients within each unit of body index is given.

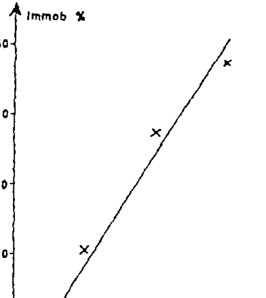


Fig 3 The relationship between number of symptoms in group 9 a (see text) and immobilization. The frequency of immobilization among patients with 1 2 or 3 of the symptoms in question is given.

Table III The relationship between number of symptoms in group 9a (see text) and 4 immobilization

No. of symptoms 9a	Total no. of pts	Immobilization 4	
		No. of pts	%
0	19	0	0
1	39	8	20.5
2	51	19	37.3
3	19	9	47.4

observed in the factor analysis and elucidated in fig 1

The relation between immobilization and low PWC is only slightly significant. Low PWC has a very strong correlation to marked OPR. There is a positive but not significant correlation between immobilization and marked OPR.

Because of the relation between low PWC and marked OPR the associations between immobilization and low PWC have been calculated separately for the groups with marked and normal OPR and have been found to be + 0 15 and — 0.18 respectively None of these values is significant and the mean value for the association in the whole material in homogeneous groups with regard to OPR is very close to 0 This strongly indicates that there is no true correlation between immobilization and low PWC.

Immobilization has its strongest correlation to factor 9 a 2 or 3 of the symptoms palpitations, breathlessness and anxiety The relationship between immobilization and these symptoms is further elucidated in table III where factor 9 a is divided into number of symptoms. In fig 3 the frequency of immobilization is given as a function of number of symptoms, and the relationship is almost a straight line. With none of these symptoms, i. e when only precordial pain is present, no patient was immobilized, which is noteworthy

Analysis of the influence of bodily constitution, sex and age

When discussing orthostatic reactions, bodily constitution must be considered, as a slender body gives rise to marked pooling of the blood in the standing position and, thus, marked orthostatic reactions from dimensional causes.

Table IV. Association coefficients between sex, age and body index, and the associations to the main components in the condition NCA, observed by factor analysis (see also text)

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Factor analysis has shown that there are three main components constituting the

condition NCA one comprising the subjective symptoms studied — palpitations, breathlessness and anxiety but excepting precordial pain and two groups of objective components one of which is best represented by sympathicotonic ECG changes after work "ECG component and the other by marked OPR, "high pulse rate component Among the subjective symptoms palpitations are significantly positively correlated to ECG changes after work and low PWC (= high pulse rate during work)

Precordial pain on the contrary is either not or negatively correlated to other studied symptoms and signs The nature of the precordial pain is obscure but the lack of significant correlations to ECG changes and high pulse rate component further stresses that it has nothing in common with ischaemic heart disease and that it probably is not caused by vasomotor disturbances. The precordial pain may be correlated to disturbances not investigated in this paper e. g breathing abnormalities, as suggested by Friedman (1) Indeed precordial pain has been observed and provoked in correlation to abnormal breathing (9)

Another possible explanation for the divergent correlations of precordial pain is that the material is selected and includes relatively too many patients with precordial pain, because this symptom more than other ones brings the patient to see a doctor This group with precordial pain as the only symptom may thus represent an abortive type of the disease. The selection is a difficult problem in a case material like this, where the patients are investigated because of subjective symptoms, which are to a certain degree now and then evoked also in normal people. The demarcation from a healthy normal material is difficult to draw and requires an analogous analysis of a healthy normal material.

Experience with normal material in Denmark (3) and in the Framingham study (4) has given a frequency of about 20 % of NCA symptoms, but no increased incidence of pathological ECG changes at rest as compared with symptom-free people The ECG findings in NCA may change rapidly and in the present papers the investigations are often repeated and the most abnormal findings reported. The materials are therefore not comparable

Low PWC Immobilization may in itself be suspected to cause low PWC (high pulse rate at work) and marked OPR. There is a positive but not significant correlation between immobilization and marked orthostatic reactions. There is a slightly significant positive correlation between immobilization and low PWC, but when the material is divided in groups which are homogeneous with regard to OPR, no correlation is to be found Immobilization therefore cannot be the dominating cause of low PWC. Low PWC shows a stronger and highly significant positive correlation to marked OPR but as some of the patients have performed the work test even in the lying position and even then had low PWC, marked orthostatic pooling of the blood can hardly mediate the low PWC. *Low body index* is slightly positively correlated to marked OPR which is to be expected from dimensional causes in every case material whether healthy or diseased Body index is, however not correlated to low PWC and hence the strong correlation between OPR and low PWC seems to be mediated by a different factor

The essential common feature in low PWC and marked OPR is a high pulse rate Whether this is due primarily to

increased sensitivity of the vasomotor centre or to very strong impulses to it, it is not possible to say. Anyhow the low PWC and marked OPR seem to be manifestations of the wide-spread vasomotor disturbance and caused by the condition NCA per se, as is further discussed in paper III in this series.

Immobilization is strongly correlated to the number of symptoms in the condition NCA, and seems to be a function of them (table III fig 3). This is well in accordance with earlier published observations (5) and seems to indicate that in NCA as in other diseases the subjective symptoms cause the immobilization, not the reverse.

Summary

Statistical analyses of an earlier reported case material of 128 patients with neurocirculatory asthenia (NCA) has been performed with regard to some important symptoms and signs in work test and orthostatic test.

Association coefficients and factor analyses of these coefficients have indicated that three main groups of symptoms and signs constitute the condition NCA 1) the subjective symptoms palpitations, breathlessness and anxiety) ECG changes at rest and after work and 3) marked orthostatic pulse reaction (OPR) and low physical working capacity (PWC)

Palpitations but not precordial pain are positively correlated to ECG changes after work and low PWC. This indicates that precordial pain is caused by disturbances other than vegetative, vasomotor ones. Further analyses showed that immobilization does not in itself bring about low PWC. Immobilization is mainly a function of the number of the subjective symptoms.

There is a highly significant positive correlation between low PWC and marked OPR. Low body index, corresponding to a slender body is slightly significantly correlated to marked OPR but not to low PWC. The presence of low PWC even in the lying position shows that marked pooling of the blood is not the main cause of the low PWC. The essential common feature in low PWC and marked OPR is a high pulse rate, which seems to be manifestation of the wide-spread vasomotor disturbance in the condition NCA.

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From the Department of Internal Medicine (Head. H Lagerlöf, M. D) and the King Gustaf V Research Institute (Head. G Birke M. D) Karolinska Institute, Stockholm, Sweden

Implications of the Total Erythrocyte Production in Man on Erythroblast Generation Times

By

Peter G Reizenstein

To understand the nature of leukemia, knowledge of the proliferation rate of normal bone marrow cells is essential. Much work on the proliferation of bone marrow cells has resulted in quite few definite estimates (3, 9 12, 16, 19 27) of the human red cell precursor generation times (i. e., sum of interkinetic and mitotic times) The methods and results of the previous work are reviewed in table I None of them considers the final result of erythropoiesis — the total red cell production — and to do so is the purpose of the present work.

Assumptions

The assumptions serve only to define the conditions of analysis. The effect of conditions at variance with the assumptions will be discussed.

) Between the earliest recognized red cell precursor and the reticulocyte, there are four morphologically distinguishable stages, called procrythroblasts (P) basophilic (B), medium or polychromatic (M) and orthochromatic (O) erythroblasts. At the end of each stage except O one division is assumed.

b) Intra-marrow cell death and maturation without division are ignored.

c) The earliest of the recognizable red cell precursors is self-perpetuating

d) The erythropoietic-regulated "feed into the earliest recognizable red-cell-precursor compartment is of importance mainly under conditions of accelerated erythropoiesis (7 12 23)

) Only in proerythroblasts do hemi-homoplastic mitoses occur where daughter cells do not differentiate All other erythroblast mitoses are assumed to be heteroplastic.

Additional information used

f) Total ed cell production. Five types of data are available and are reviewed in table II An verage total production of 2 0 × 10^{11} erythrocytes per day in a 60 kg man was used in the calculations.

g) Number of red cell precursors. The total verage normal bone marrow volume in man (table III) and the cell distribution in the bone marrow (1) were used to estimate the total number of red cell precursors. An average total of 10.0 × 10^{11} bone marrow cells in a 60 kg man was used in the calculations. The distribution of erythroblast maturation stages is given in table IV

Calculations

Let N = no. of cells in compartment indicated by subscript,

t = generation time in days,

then $\frac{N}{t}$ = no. of new cells produced, P

According to assumption c,

$$P_P = \frac{N_P}{t_P} \quad (1)$$

In a steady state, this must be the no. of cells entering compartment B

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f) Total red cell production. Five types of data are available and are reviewed in table II. An average total production of 2.0 × 10^{11} erythrocytes per day in 60 kg man was used in the calculations.

g) Number of red cell precursors. The total average normal bone marrow volume in man (table III) and the cell distribution in the bone marrow (1) were used to estimate the total number of red cell precursors. An average total of 10.0 × 10^{9} bone marrow cells in 60 kg man was used in the calculations. The distribution of erythroblast maturation stages is given in table IV.

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Table I Estimates of human red cell precursor generation times

Author and reference	Method	Generation time (hours)		
		tp	tB	tM
Astaldi et al. (3)	Tissue culture	–	18–21	12–24
Weicker (26–27)	Regeneration rat after plastic crisis or in pernicious anemia	24	24	24
Lajtha et al. (12)	P[32] and C[14]-adenine labeling in tissue culture	20	20	30
Killmann et al. (9)	Mitotic indices	19.8–39.6	13.6–16.3	10.8–11.5
Patt (16)	Mitotic indices	5.8	–	–
Pollycove et al. (17)	Fe^{59}-labeling	"Mean erythron hemoglobinization time" 1.4 days		
Rosenstein et al. (19)	H^3-thymidine	3.2	7.3	23
Present report	Total red cell production	2.4	11.3	28

*Proliferating class   *Turnover times

Table II Total red cell production according to different methods

Reference	Method	Average erythrocyte life span (days)	Resultant red cell production/day
10	Carbon monoxide or bilirubin production	–	2.11 10
6 14	Isotop nitrogen	115	2.13 10
17	Isotop iron	118	2.08 10
5	Diisopropylfluorophosphate (DFP)	125	1.97 10
13	Differential agglutination according to Ashby	120	2.04 10
	Value used in calculations	–	2.0 10

Weighted mean bilirubin production 240 mg (56 patients) corresponding to breakdown of 6.55 g hemoglobin daily (10) and 30 μμg hemoglobin per red cell.
Calculated for 60 kg man with 2.43 10 red cells.

Table III Estimates of total bone marrow cellularity

Reference	Method	Total no. bone marrow cells
8	Radio-iron	10.9 10
24	Radio-iron	9.9 10
16	Mitotic indices	10.4 10
	Average	10.0 10

Calculated for 60 kg man.

Table IV Erythroblast percentages. As computed by Albritton (1) from values collected by 21 authors from 750 adults. The large variation is obvious and probably due to the poor definition of cells which is the basic problem in analysis of this kind

	P	B	M	O
Mean	0.5	2.4	11	4.5
Range	0.2–[illegible]	1.[illegible]–[illegible]	5.0–26.4	1.6–21.5

E_B Hence

$$E_B = \frac{N_P}{t_P} \qquad (2)$$

$$P_B = \frac{\lambda_B}{t_B} \qquad (3)$$

According to assumption c,

$$E_M = \frac{2\lambda_B}{t_B} \qquad (4)$$

for not only one but both daughter cells leave B Hence, in a steady state

$$E_M = P_B + E_B \qquad (5)$$

Similarly

$$E_O = \frac{2N_M}{t_M} \text{ and} \qquad {}^1(6)$$

$$E_O = P_M + E_M \qquad (7)$$

According to assumption a the no. of erythrocytes entering circulation in a steady state

$$E_E = E_O \qquad (8)$$

From table I

$$E_E = 2.0 \times 10^{11} \qquad (9)$$

Two pairs of equations are thus easily obtained

$$\begin{cases} E_O = \dfrac{\lambda_M}{t_M} + \dfrac{2N_B}{t_B} & (4 \text{ and } 7) \\ E_O = \dfrac{2\lambda_M}{t_M} & (6) \end{cases}$$

$$\begin{cases} E_M = \dfrac{\lambda_B}{t_B} + \dfrac{N_P}{t_P} & (2,\ 3,\ 5) \\ E_M = \dfrac{2\lambda_B}{t_B} & (4) \end{cases}$$

Whence

$$\frac{\lambda_M}{t_M} = \frac{2\lambda_B}{t_B} \qquad (10)$$

$$\frac{\lambda_B}{t_B} = \frac{\lambda_P}{t_P} \qquad (11)$$

Since E_O is known (eq 8 and 9) and average λ s can be taken from tables III and IV the generation times can be obtained from equations 9 10 and 11

$$t_M = \frac{2\lambda_M}{E_O} = 1.17 \text{ days} \qquad (6)$$

$$t_B = \frac{2N_B \cdot t_M}{\lambda_M} = 0.48 \text{ days} \qquad (10)$$

$$t_P = \frac{\lambda_P \cdot t_B}{\lambda_B} = 0.10 \text{ days} \qquad (11)$$

This equation has been given previously by Cronkite et al. (7)

Discussion of method

None of the methods used to estimate generation times is perfect.

The tissue culture and cytocinematographic methods may be suspected of so changing the cellular surroundings, that time parameters are also changed.

The regeneration in pernicious anemia has not been shown to occur at physiologic proliferation rates.

The radiation from DNA labeling could influence cell growth grain counts may be influenced by local blood supply and the rate of grain count diminution may be influenced by the flux of labeled cells between morphologic compartments. The analysis of labeling index variations (19) suffers from the limited number of cells and subjects studied.

To calculate generation times from mitotic indices, knowledge of mitotic times is required and assumptions in this respect are uncertain.

The present method finally suffers from the wide variations in the basic figures (table III) When the generation times were calculated for all maximum, mean and minimum erythroblast percentages in different combinations (table III) the range of values obtained was so wide as to render them meaningless. This fact is mentioned since it bears on the validity of the means given in table IV and to demonstrate that a wide range of generation times is compatible with a reasonable red cell production. It is only for the medium (polychromatic) erythroblasts that conditions are sufficiently restricted that it can be shown what generation times agree with the known red cell production (fig 1) To get better morphologic definitions of cells, and to establish bone marrow differential counts should be one major objective of future work.

Assumptions c, d and e

If the *P-compartment* is not self-perpetuating but all its mitoses are heteroplastic as assumed by Lajtha (12) then

$$E_S = \frac{2N_P}{t_P}$$

and $t_P = 4.8$ hours. Lajtha assumes that the number of stem cells $N_S = 4$ per cent of the total bone marrow or 4×10^9 stem cells. In that case,

$$t_S = \frac{2N_S}{E_P} = \frac{2N_S\ t_P}{N_P} = 1.6 \text{ days.}$$

Discussion of results

The present results are in agreement only with some of those previously reported (table I). All results quoted (table I) except one (9) are compatible with a generation time between 20 and 30 hours for medium or polychromatic (*M*) erythroblasts. Since this is the last dividing compartment, its output will determine the resultant red cell production, and there is good agreement between the time 20 to 30 hours, the average medium erythroblast percentage, and the known red cell production (fig. 1).

There is less agreement where the proerythroblast is concerned. The present figure of 2.4 hours is compatible with some of those previously found (16, 17, 19). Lajtha et al. (12), Weicker (27) and Killman et al. (9), on the other hand, report a generation time of 20 to 40 hours and the tissue culture studies of Boll et al. (4) are compatible with such a time.

This latter figure is as long as, or longer than, that reported for the more mature cells. This is an unlikely state, for there are many indications that the immature erythroblasts grow more rapidly than the mature ones (20, 21, 25). The incompatibility of the data suggesting (4) or assuming (9) mitotic times of about 1 hour and the present data may in fact, suggest more than 1 mitosis per maturation stage.

A more serious argument against the long proerythroblast generation time is the following:

$$\frac{N_P}{N_B} = \frac{t_P}{t_B} \qquad (11)$$

$$\frac{N_B}{N_M} = \frac{t_B}{2t_M} \qquad (10)$$

According to table IV

$$\frac{N_P}{N_B} \approx \frac{N_B}{N_M} \approx \frac{1}{5}$$

whence

$$12.5\ t_P = t_M$$

It is thus unlikely that $t_P = t_M$, and even more that $t_P > t_M$ (9).

Summary

Previous estimates of red cell precursor generation times are reviewed. A new estimate is made on the basis of known data on total red cell production and bone marrow volume. Generation times of 2.4 hours for proerythroblasts, 11.3 hours for basophilic erythroblasts, and 28 hours for medium or polychromatic erythroblasts were found. The latter time tallies well with all previous estimates except one.

It is shown that previous estimates of 20—40 hours for proerythroblasts are not easily compatible with known data. This incompatibility may suggest several mitoses per maturation stage.

The present work focuses attention on the two major areas where our present

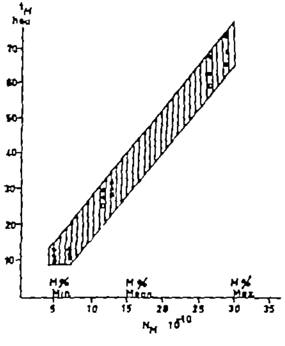


Fig 1 In the present paper the generation time of the last dividing erythrocyte precursor (t_M) is calculated on the basis of data on

1) (Bone marrow volume) × (M) − λ_M and

2) Red cell production.

However each of these parameters varies within an appreciable range and the time calculated is that obtained by using the mean of each parameter If instead of the mean, maximum or minimum values are used, widely differing results are obtained. This is what is illustrated by the present figure, which may be said to show the values for t_M compatible with the most extreme combinations of other parameters.

It is seen, that t is the percentage of medium or polychromatic erythroblasts ("percent M") which causes the widest variation. Estimates of the other parameters vary less.

Symbols	Bone marrow volume (table III)	Red cell production (*table II*)
○	Minimum	Minimum
●	Minimum	Mean
□	Minimum	Maximum
△	Maximum	Minimum
▲	Maximum	Mean
■	Maximum	Maximum

Discussion of the assumptions

In all previous studies assumptions were made, consciously or unconsciously and more or less like those made here. To establish more firmly the model to select — the origin of the proerythroblasts, the number of cell divisions, the extent of intra marrow cell death and "maturation without-division" the type of mitosis (homo- hetero- or hemihemoplastic) should be the other major object for future work. Once this is done, the generation times can be calculated by simple methods like the present ones.

Only a few of the alternatives to the assumptions made can be discussed

Assumption a

Most authors agree on 3 divisions during the development from proerythroblast to erythrocyte (12) and divide erythrocyte precursors into 4 classes (1) although both more mitoses (26) and more classes (18) are conceivable. However the demonstration of five volume classes among erythroblast nuclei by Weicker (26) does not necessarily imply five generations. Even with four generations, five nuclear classes would be expected for 2 classes of proerythroblast nuclei would be found (tetraploid after DNA synthesis but diploid after mitosis prior to new DNA synthesis) This is the reason why four generations were assumed here Nevertheless, the uncertainty in this respect is perhaps the most serious argument against the present analysis, and also against several of the previous studies.

Assumption b

If the daily percentage of cells dying in a marrow compartment is D the production will be

$$P = \frac{N\,(100-D)}{t}$$

Assumptions c, d and e

If the *P*-compartment is not self perpetuating but all its mitoses are heteroplastic as assumed by Lajtha (12) then

$$E_B = \frac{2\lambda_P}{t_P}$$

and $t_P = 4.8$ hours. Lajtha assumes that the number of stem cells $\lambda_S = 4$ per cent of the total bone marrow or 4×10^3 stem cells. In that case,

$$t_S = \frac{2\lambda_S}{E_P} = \frac{2\lambda_S}{\lambda_P}\frac{t_P}{} = 1.6 \text{ days.}$$

Discussion of results

The present results are in agreement only with some of those previously reported (table I). All results quoted (table I) except one (9) are compatible with a generation time between 20 and 30 hours for medium or polychromatic (M) erythroblasts. Since this is the last dividing compartment, its output will determine the resultant red cell production, and there is good agreement between the time 20 to 30 hours, the average medium erythroblast percentage, and the known red cell production (fig. 1).

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The present work focuses attention on the two major areas where our present

knowledge is insufficient. The morphologic criteria and frequency of the different erythroblast maturation stages and the number and type of divisions in each of these stages. It is on these questions future work should be concentrated. Once they are answered the calculation of the time parameters can be done according to the method outlined here.

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Let total marrow (dog) = 7.6 × 10^{9} cells/kg = body weight = 7 kg, and % M = 12.5 then N_m = 6.65 × 10^{9} "medium erythroblasts.

Let blood volume = 0.85 body weight RBC = 6.6 mill., red cell life span 95 days, then total red cell production = E_o (dog) = 41.2 × 10^{9} cells/day Whence,

$$t_m = \frac{2N_m}{E_o} = 0.322 \text{ days} = 7.8 \text{ hours.}$$

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From Medical Department B (Head: Erik Warburg, M. D.) Rigshospitalet, Copenhagen, Denmark

Hypokalaemia, Hypochloraemia, and Baseosis in Long term Treatment of Oedematous Heart Failure with Benzothiadiazine Diuretics

I. Incidence and Pathophysiology

By

Erik Sandøe and Knud Olesen

The oral non-mercurial diuretics of the benzothiadiazine group have been coming into ever increasing clinical use in recent years. Chemically they are characterized by containing a combined benzene and thiadiazine ring with a sulphamyl group in the 7 position and therapeutically by exerting a diuretic effect after oral administration and a tendency to induce hypokalaemia, hypochloraemia, and metabolic baseosis (alkalosis) (1 2, 8, 9 10 12, 13, 20, 30) Examples of the most widely used preparations and their structural formulae are given in fig. 1

The object of the present study was to elucidate the incidence and severity of hypokalaemia, hypochloraemia, and baseosis during benzothiadiazine therapy and to study the mechanism in the development of these electrolyte disturbances. We were interested particularly in the findings during long-term therapy of oedematous heart failure and based our analyses on our experience of the drug cyclopenthiazide (Navidrex)

Previous investigations (3, 4 31) have shown that cyclopenthiazide is an effective oral saluretic. A maximum diuretic effect is obtained by a daily dosage of 0.5—1 mg Like hydrochlorothiazide, hydroflumethiazide, benzhydroflumethiazide, and trichloromethiazide — and partially in contrast to chlorothiazide — cyclopenthiazide has only a slight carbonic anhydrase inhibitor effect (3 4)

Material and method

The material comprises adult patients with oedematous heart failure of rheumatic, arteriosclerotic, or congenital origin.

The treatment consisted in oral administration of cyclopenthiazide (Navidrex) 1 mg daily The majority of the patients received a full diet, and digitalis medication, if already instituted, was continued.

Submitted for publication May 22, 1962.

knowledge is insufficient. The morphologic criteria and frequency of the different erythroblast maturation stages, and the number and type of divisions in each of these stages. It is on these questions future work should be concentrated. Once they are answered the calculation of the time-parameters can be done according to the method outlined here.

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Table II Incidence of serum electrolyte disturbances in 18 patients with cardiac oedema after one month on cyclopenthiazide

Hypokalaemia (< 3.6 mEq/l)	39%
Metabolic baseosis (> 25.6 mEq/l)	50%
Hypochloraemia (< 99 mEq/l)	77%
Hyponatraemia (< 134 mEq/l)	0%

Table III Average distribution of extracellular and intracellular loss of potassium in 6 metabolic balance studies. The extracellular loss of potassium was calculated on the basis of the decrease in body weight and in serum potassium

Total potassium balance	−186 mEq
Extracellular potassium balance	− 22 mEq
Intracellular potassium balance	−164 mEq

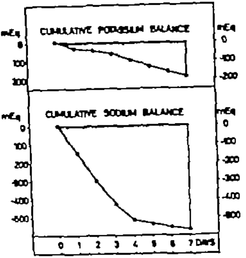


Fig. 2. Mean of metabolic balance experiments on 6 patients with cardiac oedema treated with cyclopenthiazide.

Results

Serum electrolytes

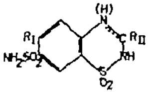
The average serum electrolyte values in 18 patients before and after one month's treatment are shown in table I Serum sodium remained unchanged throughout the treatment, while a significant fall in serum potassium and serum chloride occurred and, as a sign of metabolic baseosis, a significant increase in standard bicarbonate. p CO rose from an initial value in the low normal range to an ultimate value in the middle of the normal range, so that the pH remained unchanged. The actual bicarbonate rose to the extent that the serum chloride decreased.

Assessed on the basis of the normal values reported by our Laboratory the long-term therapy resulted in the development of hypokalaemia in 39 % of the patients, hypochloraemia in 77 %, and metabolic baseosis in 50 % (table II) Hyponatraemia was not observed and no extreme serum electrolyte disturbances occurred. The lowest serum potassium was 2.7 mEq/l, the lowest serum chloride 92 mEq/l, and the highest standard bicarbonate 27.8 mEq/l. No changes in serum creatinin occurred during the treatment.

The alterations in the serum electrolyte pattern after one week treatment showed the same tendencies as after one month treatment.

Metabolic balance experiments

The mean cumulative potassium and sodium balance from 6 experiments is given in fig 2 It may be seen that cyclopenthiazide therapy did not only entail a negative sodium balance, but also a considerable loss of potassium. During the first week of treatment the potassium deficit amounted to 186 milliequivalents. Calculated from alterations in serum potassium and body weight, the loss of extracellular potassium amounted to only 22 milliequivalents. The remaining 164 milliequivalents must represent a loss of intracellular potassium (table III)



Generic name	R_I	R_{II}
Clorothiazide	Cl	H
Hydrochlorothiazide	Cl	$-H_2$
Hydroflumethiazide	F_3C	H_2
Benzhydroflumethiazide	F_3C	$<^{H}_{CH_2}-\bigcirc$
Trichloromethiazide	Cl –	$<^{H}_{CHCl_2}$
Cyclopenthiazide	Cl –	$<^{H}_{CH_2}-\square$

Fig 1 Examples of benzothiadiazin diuretics and their chemical structure At the top the combined benzene and thiadiazine ring which is a component of all the substances. The figure gives the composition of the radicals R_I and R_{II} which vary from substance to substance

The investigation comprises long-term experiments in which the patients were treated for a month and short term experiments in which the treatment was carried out for only one week. In the long-term experiments body weight, arterial pH, p CO_2, standard bicarbonate, serum sodium, potassium, chloride, and creatinine were determined before the treatment was started and after it was completed Short term experiments were instituted by an observation period of 2—3 days and continued by one week's medication. During the entire period the daily renal excretion of water sodium, chloride, potassium, and net acid, was measured, and at the commencement and completion of the medication the body weight, arterial pH, p CO_2, standard bicarbonate, serum sodium, potassium, chloride, and creatinine were determined. The effect of cyclopenthiazide upon the renal excretion of water and electrolytes was assessed on the basis of the daily excretion increment which was calculated as daily renal excretion during the treatment week less the daily renal excretion during the observation period.

In a small group of patients the sodium and potassium balance was measured. They were given a diet with a known content of sodium, potassium and nitrogen, and the electrolyte balance was calculated as the difference between the intake and excretion in the urine and faeces. In order to rule out the effect of protein ana and catabolism, the potassium balance was corrected to nitrogen equilibrium on the basis of a nitrogen- potassium ratio of 1 g 3 mEq

The chemical analyses were carried out as routine analyses at the Central Laboratory of Rigshospitalet. Arterial pH, p CO_2, and standard bicarbonate were measured by methods advocated by Jorgensen and Astrup (15) and by Siggaard Andersen et al. (28). The urinary net acid was measured by the method of Jorgensen (14)

The diets were furnished by the diet kitchen of Medical Department A.

The significance analyses on the experimental results were based on Student's t test.

Table I Mean values for serum electrolytes in 18 patients with cardiac oedema treated for a month with cyclopenthiazide

Determinations	Units	Before treatment	After treatment	Difference	Significance of difference
Serum sodium	mEq/l	139.8	139.7	−0.1	Not sign.
Serum chloride	mEq/l	102.2	96.9	−5.3	$p < 0.001$
Serum potassium	mEq/l	4.24	3.66	−0.58	$p < 0.005$
Standard bicarbonate	mEq/l	22.6	25.8	+3.2	$p < 0.05$
Arterial pH		7.41	7.42	+0.01	Not sign.
p CO_2	mm Hg	35.2	39.7	+4.5	Not sign.
Serum creatinine	mg/100 ml	1.0	1.0	0	Not sign.

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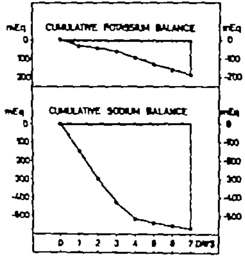


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Table IV Renal excretion of water and electrolytes Mean values for 21 patients with cardiac oedema treated with cyclopenthiazide for one week

Determination	Units	24-hour excretion		Excretion increment in the 24 hours	Significance of excretion increment
		Observation period	Treatment period		
		Mean of 3 days	Mean of 7 days		
Water	ml	769	1257	+ 488	$p < 0.001$
Sodium	mEq	74	132	+ 58	$p < 0.005$
Chloride	mEq	77	136	+ 59	$p < 0.005$
Potassium	mEq	37	61	+ 24	$p < 0.001$
Net acid	mEq	41	42	+ 1	Not sign.

Table V Mean increase in the excretion of potassium and sodium in relation to weight loss in 21 patients with cardiac oedema treated with cyclopenthiazide for one week

	Weight loss after 1 week	
	< 2 kg	> 2 kg
Increase in potassium excretion (mEq/24 hours)	+ 10	+ 35
Increase in sodium excretion (mEq/24 hours)	+ 37	+ 73
Number of patients	9	12

Renal electrolyte excretion

Table IV shows the mean daily excretion of water and electrolytes during the observation and treatment periods, calculated as the mean of 21 weeks. The treatment entailed a significant increase in the excretion of water sodium, chloride, and potassium while the excretion of net acid did not alter The increases in the chloride and sodium excretion were equivalent.

The increase in potassium excretion varied within wide limits. In table V it is related to the weight loss. It will be seen that the loss of potassium was at a maximum when the weight loss was great. The difference in the potassium excretion increment between the groups obtaining a weight loss of over and under 2 kg was significant ($p < 0.025$)

Discussion

Continuous treatment with oral benzothiadiazine diuretics involves a risk of developing hypokalaemia hypochloraemia, and baseosis. This risk has previously been demonstrated during treatment with chlorothiazide (9 11 20 22) hydrochlorothiazide (2, 8, 18, 30) hydroflumethiazide (10 19) benzhydroflumethiazide (1 2 30) and trichloromethiazide (12) and as demonstrated above it is also present during the use of cyclopenthiazide (4 31) The same applies to chlorothalidone (Hygroton) (25 29)

The fall of serum potassium reflects a renal loss of potassium which does not merely reduce the extracellular potassium but gives an even more marked reduction of the potassium content in the cells. This is demonstrated by our balance studies and has been confirmed by Hollander et al (11) who found that the body content of total exchangeable potassium fell by 5—10 % during long

term treatment with chlorothiazide. Therefore, even a slight hypokalaemia should be regarded with some misgivings, as it may be associated with a considerable intracellular potassium deficiency

The renal excess excretion of potassium which conditions the extracellular as well as the intracellular loss of potassium varies from patient to patient. In our experiments the loss of potassium was significantly greater in patients who lost much weight than in those who did not benefit from the treatment. Thus, we cannot confirm the tendency to a greater loss of potassium in patients who give a poor response to the treatment as has been reported by others (13 29)

The development of hypochloraemia is due to the fact that the renal excess excretion of chloride and sodium occurs in equivalent amounts. In the extra cellular phase the ratio chloride sodium will be 0.8 1 and a renal excess excretion at the ratio 1 1 therefore, entails a loss of chloride in relation to sodium, thus resulting in hypochloraemia.

As demonstrated in our experiments the basesis is of metabolic nature, and it is compensated for by respiratory means, the p CO from the subnormal level which is characteristic of dyspnoeic patients in heart failure (25) increasing to values in the middle of the normal range. The increase in actual bicarbonate corresponds to the decrease in serum chloride, which might be interpreted as a sign of so-called hypochloraemic baseosis This relation between hypochloraemia and baseosis is not obligatory however as evident from the fact that a supplement of potassium chloride can effectively reduce the tendency to hypochloraemia without affecting the baseosis (26) and that a supplement of

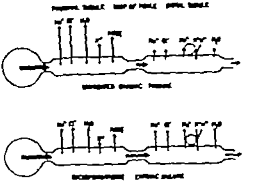


Fig 3. Effect of benzothiadiazines on the renal transport of electrolytes.

spironolactone eliminates the baseosis without essentially affecting the hypochloraemia (24)

Like hypokalaemia and hypochloraemia the baseosis is presumably also of renal origin. The mechanism of its development is presumably a renal loss of hydrogen ions. That its development is not associated with an increase in urinary net acid may be due to the fact that the patients are being dehydrated at the same time and therefore have free bicarbonate from the excreted oedema fluid at their disposal. In the presence of an increased bicarbonate excretion the lack of change in net acid excretion (= titratable acid + ammonium — bicarbonate) may be taken as evidence of an increased renal excretion of hydrogen ions. A contributory factor in the genesis of baseosis may be internal re-distribution of hydrogen ions with an afflux of these ions to the cells to make up for the lost potassium and subsequent development of extracellular baseosis (6) Our experiments using a supplement of potassium chloride, however have shown that the maintenance of the potassium balance does not prevent the development of baseosis and thus do not confirm this hypothesis (26)

Table IV Renal excretion of water and electrolytes Mean values for 21 patients with cardiac oedema treated with cyclopenthiazide for one week

Determination	Units	24-hour excretion		Excretion increment in the 24 hours	Significance of excretion increment
		Observation period	Treatment period		
		Mean of 3 days	Mean of 7 days		
Water	ml	769	1257	+ 488	$p < 0.001$
Sodium	mEq	74	132	+ 58	$p < 0.005$
Chloride	mEq	77	136	+ 59	$p < 0.005$
Potassium	mEq	37	61	+ 24	$p < 0.001$
Net acid	mEq	41	42	+ 1	Not sign.

Table V Mean increase in the excretion of potassium and sodium in relation to weight loss in 21 patients with cardiac oedema treated with cyclopenthiazide for one week

	Weight loss after 1 week	
	< 2 kg	> 2 kg
Increase in potassium excretion (mEq/24 hours)	+ 10	+ 35
Increase in sodium excretion (mEq/24 hours)	+ 37	+ 73
Number of patients	9	12

Renal electrolyte excretion

Table IV shows the mean daily excretion of water and electrolytes during the observation and treatment periods, calculated as the mean of 21 weeks. The treatment entailed a significant increase in the excretion of water sodium, chloride, and potassium while the excretion of net acid did not alter The increases in the chloride and sodium excretion were equivalent.

The increase in potassium excretion varied within wide limits. In table V it is related to the weight loss. It will be seen that the loss of potassium was at a maximum when the weight loss was great. The difference in the potassium excretion increment between the groups obtaining a weight loss of over and under 2 kg was significant ($p < 0.025$)

Discussion

Continuous treatment with oral benzothiadiazine diuretics involves a risk of developing hypokalaemia, hypochloraemia, and baseosis. This risk has previously been demonstrated during treatment with chlorothiazide (9 11 20 22) hydrochlorothiazide (2 8 18 30) hydroflumethiazide (10 19) benzhydroflumethiazide (1 2 30) and trichloromethiazide (12) and as demonstrated above it is also present during the use of cyclopenthiazide (4 31) The same applies to chlorothalidone (Hygroton) (25 29)

The fall of serum potassium reflects a renal loss of potassium which does not merely reduce the extracellular potassium but gives an even more marked reduction of the potassium content in the cells. This is demonstrated by our balance studies and has been confirmed by Hollander et al. (11) who found that the body content of total exchangeable potassium fell by 5—10 % during long

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Pathophysiologically the renal excess excretion of potassium and the probable excess excretion of hydrogen ion may be explained by fairly simple mechanisms (fig 3) Both ions are excreted in the distal segment of the nephron and the excretion takes place in the presence of re-absorption of sodium by the so-called ion exchange mechanism (17) The activity of this mechanism is governed by the aldosterone secretion while at the same time its capacity is restricted by the extent of the sodium supply In oedematous cardiac failure the supply of sodium to the distal segment of the nephron is often slight, and the possibilities of an exchange with potassium and hydrogen ions correspondingly small. Benzothiadiazine diuretics inhibit the re-absorption of sodium in the proximal segment of the nephron (16 33) and thereby increase the supply to the distal segment (5) At the same time the patients are often in a state of hyperaldosteronism, either as a result of their cardiac failure (7 27 34) or in response to the dehydrating therapy (21 32) This situation creates ideal conditions for the ion exchange and results in a renal excess excretion of potassium and hydrogen ions which in the long run leads to hypokalaemia and metabolic baseosis.

Summary

In a series of patients with oedematous heart failure, the serum electrolyte pattern was studied during long term treatment with the benzothiadiazine derivative cyclopenthiazide (Navidrex) Treatment for one month resulted in the development of hypokalaemia, hypochloraemia and metabolic baseosis in a large proportion of the cases.

Measurements of the renal excretion of electrolytes before and during the first week of treatment showed that the hypokalaemia was due to an increase in renal excretion of potassium and that the hypochloraemia was due to an increase in the renal excretion of chloride in relation to sodium. The metabolic baseosis appears to be conditioned by an increase in the renal excretion of hydrogen ions.

In metabolic balance experiments, the negative potassium balance was far greater than the calculated extracellular loss of potassium. Therefore, cyclopenthiazide therapy must also result in a considerable intracellular potassium deficiency The loss of potassium varied, but on the average it was greatest in patients showing a marked weight loss.

Pathophysiologically the renal excess excretion of potassium and hydrogen ion is explained by an increased supply of sodium to the distal segment of the nephron combined with hyperaldosteronism.

Acknowledgement

Our thanks are due to Dr J Lorenzen of Ciba Ltd who supplied us with cyclopenthiazide (Navidrex) for the experiments reported in this and the two following papers.

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Hypokalaemia, Hypochloraemia, and Baseosis in Long term Treatment of Oedematous Heart Failure with Benzothiadiazine Diuretics

II. Prophylactic Value of Potassium Chloride

By

Erik Sandoe and Knud Olesen

The hypokalaemia which occurs during treatment with benzothiadiazine derivatives is caused by a negative potassium balance (2, 3 4 7 10, 11 12) and accordingly most investigators have found that the decrease in serum potassium may be prevented by a supplement of potassium chloride (1 5 8, 9 13 14)

In the present study this important detail of oral diuretic therapy was taken up for renewed consideration with a particular view to the prophylactic value of a supplement of potassium chloride, in treating not only the hypokalaemia, but also the hypochloraemia and metabolic baseosis (alkalosis) W were interested particularly in the findings during long-term treatment of oedematous heart failure and based our considerations on our experience of the benzothiadiazine cyclopenthiazide (Navidrex)

Submitted for publication May 22, 1962.

Material and method

The material comprises adult patients with oedematous heart failure of rheumatic, arteriosclerotic, or congenital nature.

The treatment consisted in daily oral administration of cyclopenthiazide, 1 mg, and potassium chloride, 3 g. The potassium salt was administered in the form of enterosolubl potassium chloride tablets of 1 g potassium chloride 1 tablet 3 times daily This dosage corresponds to 40 milliequivalents of potassium and 40 milliequivalents of chloride ion daily

Experimental technique, chemical, and statistical methods were the same as described in the preceding paper (12)

The investigations comprised 1) Recording of the serum electrolyte pattern in 18 patients treated for one month with cyclopenthiazide and potassium chloride, and 2) metabolic balance experiments on 5 patients treated in the same way for one week.

The value of the supplement of potassium chloride was assessed in comparison with the findings in similar experiment in which the patients received cyclopenthiazide alone (12).

Side effects

The intake of 3 g potassium chloride daily seldom gave rise to difficulties. A few patients complained of mild dyspepsia. Attempts at increasing the dosage to 6 g daily on the other hand, quite often gave rise to gastrointestinal symptoms.

Discussion

According to our balance experiments it is possible, by a daily supplement of 3 g potassium chloride, to obtain natriure as on cyclopenthiazide without a simultaneous loss of potassium from the cells or the extracellular phase. Conformably there was no significant decrease in serum potassium during long-term therapy and hypokalaemia was an uncommon occurrence.

In our experiments the supplement of potassium chloride did not merely compensate for the loss of potassium, but also for the chloride loss, the administration of chloride ion preventing the development of hypochloraemia. In contrast, metabolic baseosis developed with the same frequency as in cyclopenthiazide therapy without a potassium supplement. This militates against the baseosis in cyclopenthiazide therapy bearing an obligatory relation to hypochloraemia or intracellular potassium deficiency (6 12) and indicates that the metabolic baseosis is of an independent genesis. Presumably it is due to a renal loss of hydrogen ions (12) and this loss is not compensated for by a supplement of potassium chloride.

The magnitude of the optimum potassium chloride supplement varies from patient to patient. Particular regard should be paid to patients with impaired renal function in whom even a slight extra dose may give rise to severe hyperkalaemia. In cases of normal renal function, a daily supplement of 3 g potassium chloride is usually sufficient, according to our own experience and that of others (1 5 8, 9 13) while a smaller supplement is accompanied by an increasing incidence of hypokalaemia (8). Doses of more than 3 g potassium chloride, which may be advisable in a few patients, often give rise to dyspepsia if continued for some length of time.

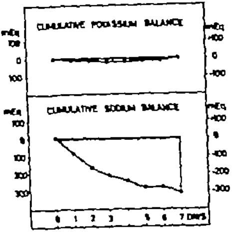


Fig. 1 Mean of metabolic balance experiments on 5 patients with cardiac oedema treated with cyclopenthiazide 1 mg and potassium chloride 3 g for one week.

Summary

In a series of patients with oedematous heart failure being treated with the benzothiadiazine derivative cyclopenthiazide (Navidrex) the effect of a daily supplement of 3 g potassium chloride was investigated. This supplement was found to maintain the potassium balance during the natriuresis, nd during long term therapy it prevented to a marked extent the development of hypokalaemia and hypochloraemia. On the other hand, the incidence of metabolic baseosis remained unaffected.

Table I Mean values for serum electrolytes in 18 patients with cardiac oedema treated for one month with cyclopenthiazide 1 mg and potassium chloride 3 g

Measurements	Units	Before treatment	After treatment	Difference	Significance of difference
Serum sodium	mEq/l	139.7	139.7	0	Not sign.
Serum chloride	mEq/l	101.6	100.1	− 1.5	Not sign.
Serum potassium	mEq/l	4.12	4.02	− 0.10	Not sign.
Standard bicarbonate	mEq/l	22.4	24.8	+ 2.4	$p < 0.05$
Arterial pH		7.40	7.42	+ 0.02	Not sign.
Arterial p CO_2	mm Hg	35.8	40.4	+ 4.6	$p < 0.02$
Serum creatinine	mg/100 ml	1.0	1.0	0	Not sign.

Table II Incidence of serum electrolyte disturbances in patients with cardiac oedema after one month on cyclopenthiazide medication with and without a supplement of potassium chloride

	Supplement of potassium chloride	
	−	+
Hypokalaemia (< 3.6 mEq/l)	39%	11%
Metabolic baseosis (>25.6 mEq/l)	50%	39
Hypochloraemia (< 99 mEq/l)	77%	39%
Hyponatraemia (<134 mEq/l)	0	6
No of patients	18	18

Results

Serum electrolytes

The average alterations in serum electrolytes in 18 patients treated for a month with cyclopenthiazide and potassium chloride are given in table I which shows that the treatment did not entail significant decreases in serum potassium or serum chloride, while the patients showed a significant increase in standard bicarbonate indicating metabolic baseosis. The arterial pH was maintained unchanged by a compensatory increase in p CO_2. In a corresponding series of 18 patients treated with cyclopenthiazide alone for one month there was a significant decrease in serum potassium and serum chloride and the same degree of metabolic baseosis (12)

The incidence of hypokalaemia hypochloraemia, and baseosis in these two series is shown in table II The supplement of potassium chloride reduced the incidence of hypokalaemia and hypochloraemia but did not affect the incidence of metabolic baseosis. Extreme serum electrolyte values were rare in both groups. The lowest serum potassium was 3.0 mEq/l with a supplement of potassium chloride and 2.7 mEq/l without, the lowest serum chloride 94 and 92 mEq/l respectively and the highest standard bicarbonate 30.5 and 27.8 mEq/l respectively

Metabolic balance experiments

Fig 1 sets out the mean potassium and sodium balance in 5 patients who were treated for one week with cyclopenthiazide and 3 g potassium chloride daily It will be seen that the treatment entailed a negative sodium balance without loss of potassium The benefit of the potassium chloride supplement is evident from the fact that corresponding experiments in which only cyclopenthiazide was given showed an average negative potassium balance of 186 milliequivalents (12)

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From Medical Department B (Head: Erik Warburg, M. D.) Rigshospitalet, Copenhagen, Denmark

Hypokalaemia, Hypochloraemia, and Baseosis in Long-term Treatment of Oedematous Heart Failure with Benzothiadiazine Diuretics

III. Effect of Spironolactone

By

Knud Olesen and Erik Sandøe

Long-term treatment with benzothiadiazine diuretics often entails hypokalaemia, hypochloraemia, and metabolic baseosis (alkalosis). In a previous paper it was emphasized that the alterations in serum electrolytes are due mainly to the fact that the natriuresis is accompanied by an unwanted renal loss of potassium, chloride, and hydrogen ions (12).

Since the renal excess excretion of potassium and hydrogen ions is due to mechanisms involving an aldosterone effect, it would seem rational to prevent these losses by combining the benzothiadiazine diuretic with an aldosterone antagonist, e.g. spironolactone (Aldactone) (1, 2, 6, 8, 11, 13). This combination also affords a chance of potentiating the diuretic effect (2, 4, 10, 16, 18, 21).

The present study was designed to investigate, in a fairly large group of patients with oedematous cardiac failure, to what extent spironolactone could prevent hypokalaemia and baseosis during continuous administration of cyclopenthiazide (Navidrex), to study the mechanism of this effect, and to assess the potentiating diuretic effect of spironolactone.

Material and method

The material comprised adult persons suffering from oedematous heart failure of rheumatic, arteriosclerotic or congenital origin.

The treatment consisted in daily oral administration of cyclopenthiazide (Navidrex) 1 mg and spironolactone (Aldactone) 400 mg.

The experimental design, chemical and statistical methods were on the lines described in a previous paper reporting the effect of cyclopenthiazide (12).

In order to elucidate the effect of spironolactone the results of the combined treatment will be compared with the findings when using cyclopenthiazide alone (12).

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Table III. 24-hour output of water and electrolytes. Mean for 18 patients with oedematous heart failure treated with spironolactone and cyclopenthiazide for one week

Determinations	Units	24-hour excretion		Excretion increment in the 24 hours	Significance of excretion increment
		Observation period	Treatment period		
		Mean of 3 days	Mean of 7 days		
Water	ml	673	1222	+ 549	$p < 0.001$
Sodium	mEq	27	112	+ 85	$p < 0.001$
Chloride	mEq	30	110	+ 80	$p < 0.001$
Potassium	mEq	40	47	+ 7	Not sign.
Net acid	mEq	40	41	+ 1	Not sign.

Table IV. Effect of spironolactone upon the renal excretion of electrolytes. 24-hour excretion in 12 patients who were treated, in varying sequence, for one week with cyclopenthiazide and for one week with cyclopenthiazide plus spironolactone

Electrolyte	Unit	Mean 24-hour excretion during treatment		Effect of spironolactone	Significance of difference
		Cyclopenthiazide (I)	Cyclopenthiazide + spironolactone (II)	(II) — (I)	
Sodium	mEq	85	134	+ 49	$p < 0.02$
Chloride	mEq	96	123	+ 27	$p < 0.10$
Potassium	mEq	59	49	— 10	$p < 0.025$
Net acid	mEq	43	33	— 10	$p < 0.03$

lactone is presented in fig. 1 which shows the mean values for 7 patients. The potassium balance has been corrected to nitrogen equilibrium in order to rule out the effect of anabolism or catabolism. The figure shows a positive potassium balance of 58 milliequivalents, while 393 milliequivalents of sodium were lost.

Renal electrolyte excretion

The mean daily excretion of water and electrolytes during the pre-treatment period and the first week of treatment, calculated as the mean of 18 experiments, is shown in table III. There is a significant increase in the excretion of water, sodium, and chloride, while the output of potassium and net acid remains unchanged.

The increase in the chloride and sodium output takes place in approximately equivalent quantities (fig. 2).

The alteration in the renal excretion pattern entailed by spironolactone is shown in table IV comparing the mean 24-hour output of electrolytes in 12 patients treated in varying sequence for a week with cyclopenthiazide alone and

Table I Mean serum electrolyte values in 22 patients treated for one month with spironolactone and cyclopenthiazide

Determinations	Units	Before treatment	After treatment	Difference	Significance of difference
Serum sodium	mEq/l	139.0	137.1	−1.9	$p < 0.05$
Serum chloride	mEq/l	102.0	97.5	−4.5	$p < 0.001$
Serum potassium	mEq/l	4.22	4.39	+0.17	Not sign.
Standard bicarbonate	mEq/l	22.5	22.9	+0.5	Not sign.
Arterial pH		7.39	7.40	+0.01	Not sign.
Arterial pCO_2	mm Hg	36.9	37.9	+1.0	Not sign.
Serum creatinine	mg/100 ml	1.0	1.0	0	Not sign.

Table II Incidence of serum electrolyte disturbances in patients with oedematous heart failure after one month on cyclopenthiazide with and without a supplement of spironolactone

	Supplement of spironolactone	
	−	+
Hypokalaemia (< 3.6 mEq/l)	39%	0%
Metabolic baseosis (> 25.6 mEq/l)	50%	10%
Hypochloraemia (< 99 mEq/l)	77%	68%
Hyponatraemia (< 134 mEq/l)	0%	23%
Number of patients	18	22

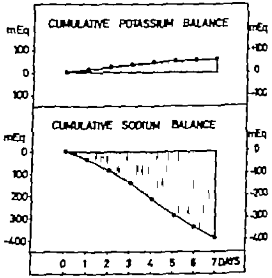


Fig 1 Mean of metabolic balance studies on 7 patients with oedematous cardiac failure treated with cyclopenthiazide and spironolactone.

Results

Serum electrolytes

Table I gives the mean values for serum electrolytes in 22 patients treated for one month with a combination of spironolactone and cyclopenthiazide. During the treatment there was a decrease of serum sodium and even more of serum chloride. On the other hand, serum potassium standard bicarbonate arterial pH arterial pCO_2, and serum creatinine remained unchanged.

The advantage obtained by a supplement of spironolactone may be seen from table II which compares the incidence of serum electrolyte anomalies following spironolactone plus cyclopenthiazide therapy with the findings with cyclopenthiazide alone. The use of spironolactone practically eliminated the hypokalaemia and metabolic baseosis, while hypochloraemia was of the same incidence in both series. A new feature was hyponatraemia in one-quarter of the patients treated with spironolactone.

Metabolic balance studies

The cumulative balance of potassium and sodium during the first week of treatment with cyclopenthiazide plus spirono-

Discussion

During the long-term therapy of cardiac oedema by a combination of cyclopenthiazide plus spironolactone the serum potassium standard bicarbonate arterial pH and pCO values remained normal, while serum chloride and serum sodium tended to fall.

The maintenance of a normal serum potassium during the long term experiments accords with the fact that the total content of potassium in the organism did not alter significantly during the first week of treatment. This was clearly evident from the metabolic balance studies as well as from the study of renal excess excretion. In other words, the investigation showed that a combination of cyclopenthiazide with spironolactone affords natriuresis without any loss of potassium. This finding which confirms the trends in previous studies (2, 8, 10 18) is in marked contrast to our own experience of cyclopenthiazide alone which involved hypokalaemia and intracellular loss of potassium owing to a renal excess excretion of potassium (12)

Standard bicarbonate in the plasma did not alter significantly during long-term treatment with cyclopenthiazid and spironolactone, and metabolic baseosis was a rare phenomenon. The explanation why the supplement of spironolactone so markedly prevents the metabolic baseosis occurring with cyclopenthiazide alone (12) must be that spironolactone inhibits the renal excretion of hydrogen ion This effect was demonstrated in the present experiments illustrated in table IV and accords also with the experience of other workers (1 8) The reason why this effect was not evident in the combined therapy experiments shown in table I was apparently

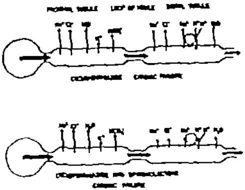


Fig 4 Effect of spironolactone upon the renal electrolyte transport during benzothiadiazine therapy

that the experimental set-up was too crude for this purpose as reflected in a marked variation in the experimental results. There is no evidence to support the alternative possibility viz. that in addition to its renal effect spironolactone might entail an internal re-distribution of hydrogen ions.

While abolishing hypokalaemia and baseosis, the spironolactone supplement does not exert any definite effect upon the hypochloraemia, but adds — as a new feature — hyponatraemia. Since the hypochloraemia is far more marked than the hyponatraemia, t can hardly be interpreted as a phenomenon attendant upon the hyponatraemia which is often seen in spironolactone therapy (13 14) As in treatment with cyclopenthiazide alone the hypochloraemia must be attributed mainly to the renal excess excretion of chloride and sodium t the ratio 1 1 which results, as emphasized previously in a deficiency of chloride in relation to sodium (12)

Fig 4 outlines the probable mechanism of the effect of the combined treatment upon the kidneys. The benzothiadiazine preparation blocks the reabsorption of

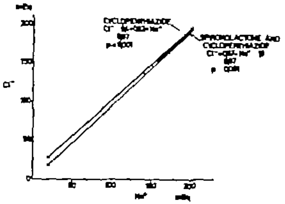


Fig 2 Relationship between excess excretion of chloride and sodium.

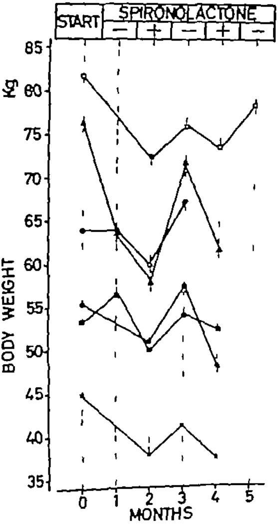


Fig 3 Examples of potentiating diuretic effect of spironolactone during long-term treatment with cyclopenthiazide.

with cyclopenthiazide plus spironolactone The effect of spironolactone manifests itself as an increased excretion of sodium and chloride and a reduced excretion of potassium and net acid

Potentiating diuretic effect of spironolactone in long term therapy

The potentiating diuretic effect attributed to spironolactone in long-term therapy was assessed in a group of 19 patients treated in varying sequence, with cyclopenthiazide for one month and with cyclopenthiazide plus spironolactone for one month. During the cyclopenthiazide medication the average weight loss was 2 1 kg During the combined therapy there was an additional weight loss averaging 3 8 kg

From a clinical point of view 10 out of the 19 patients were sufficiently treated on cyclopenthiazide alone, and these 10 patients lost only little weight during the combined therapy In the remaining 9 cases the cardiac decompensation was not relieved until the medication was supplemented by spironolactone, and the combined therapy entailed not only a marked weight loss, but also a striking clinical improvement

This potentiating diuretic effect of spironolactone is illustrated in fig 3 which presents the weight curves for 6 patients of the latter category

Side effects

During the treatment with cyclopenthiazide plus spironolactone two men had swelling and tenderness of the mammary glands, and one woman showed mild confusion and apathy Otherwise, there were no side effects worth mentioning

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sodium and thus also of chloride and water in the proximal tubules (7 19) *This results in an increased supply to the* distal segment of the nephron involving increased possibilities of reabsorption of sodium while potassium and hydrogen ion are excreted This ion exchange mechanism, which is governed by aldosterone may be stimulated not only by the increased supply of sodium but also by an elevated aldosterone level — *a link in the cardiac decompensation* (5 15 20) or in response to the use of diuretics (9 17) This entails an increased renal loss of potassium and hydrogen ions, leading to hypokalaemia and baseosis. The addition of spironolactone inhibits the aldosterone effect in the renal tubules, abolishes the renal loss of potassium and hydrogen ions, and *ensures the maintenance of a normal se*rum potassium and a normal acid base status.

In a number of patients spironolactone also entails an inhibition of the reabsorption of sodium together with chloride. This effect explains the increased excretion which *spironolactone can induce* during combined therapy and is particularly evident in patients who give a poor response to cyclopenthiazide alone. The increased excretion entails not only weight loss, but often also a striking clinical improvement In our series, this favourable effect *could* be *maintained* for several months.

Summary

Patients with oedematous cardiac failure were treated with a combination of spironolactone (*Aldactone*) and cyclopenthiazide (Navidrex) and the results are *compared* with previous findings with cyclopenthiazide alone

In experiments continued for one month the addition of spironolactone prevented the development of hypokalaemia and metabolic baseosis. It did *not essentially affect the occurrence of* hypochloraemia, and as a new feature it introduced a tendency to hyponatraemia

Investigation of the renal electrolyte excretion and metabolic balance studies during the first week of treatment revealed that the addition of spironolactone ensured natriuresis *without* the renal loss of potassium which accompanies the cyclopenthiazide effect Concurrently spironolactone appears to prevent the metabolic baseosis caused by cyclopenthiazide by blocking the renal excretion of hydrogen ion through inhibition of distal tubular ion exchange. The hypochloraemia is due predominantly to a *renal excess excretion of chloride in rela*tion to sodium as seen in treatment with cyclopenthiazide alone.

The use of spironolactone also entails an inhibition of renal tubular reabsorption of sodium *together* with *chloride* In patients who give a poor response to *cyclopenthiazide, this effect of spironolactone* often leads to a considerable weight loss and striking clinical improvement

Acknowledgement

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The Incidental Effect on the Heart of X-ray Irradiation

By

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Untoward side effects of X-ray therapy affecting unintended organs are well known. In certain cases these may be relatively harmless such as lung fibrosis, which may develop after irradiation of cervical glands. On the other hand the late results of this kind of therapy may be worse than the original disease, e. g. leukemia after irradiation of the spine of patients with M. Bekhterev and the thyroid carcinoma which may arise after X-ray treatment of an enlarged thymus. Of course it is sometimes difficult to prove a relationship between the original irradiation and its sequellae due to the long period after which the latter may arise, but a causal origin at present is more or less accepted in the above mentioned examples.

In some cases in which irradiation forms the last hope or is the only known treatment complications of this kind of therapy are not feared. However as X-ray treatment is becoming more effective and is able to produce longer lasting remissions and even cures, it seems necessary to consider its effects on surrounding tissues in general.

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On many occasions, when irradiating processes in or on the thorax, it seems hardly possible not to involve the heart. From experimental work the pathological changes of the heart due to overdosage of X rays are fairly well known and have been summarised by Whitfield and Kunkler (4) in such a way that little can be added. As damage done to the heart might be traced by the ECG it is surprising to see how few papers have been published relating to this subject. In the last years however some publications have appeared, which have drawn attention to a supposed correlation of irradiation of tumors situated in or on the chest and coronary infarction.

Whitfield and Kunkler (4) as well as Catterall and Evans (1) described transient changes in the ECG some weeks to months after irradiation of carcinoma of the breast. The damage done to the heart seems to have been of a benign character as it did not give rise to complaints and disappeared within a year' time.

Pearson (3) however described coronary occlusion, which arose 6 months

It seems clear from our experience that the risk is far less than was supposed. With a tumor dose of 6,000 R in our negroid patients no changes in the ECG could be observed which could be due to damage to the coronary vessels.

Eight of our patients were suffering from a hypertension of a sclerotic type and in 10 patients the serum cholesterol was grossly elevated (above 260 mg %). As these patients were potential candidates for coronary occlusion, it seems unlikely that irradiation favours coronary infarction.

As coronary occlusion and neoplasm are both seen usually in people above 50 years it is possible that above mentioned authors were so unfortunate as to encounter both diseases in one patient. From our systematic approach to the problem, we are inclined to think that the occurrence of a coronary infarction as an accident of irradiation is pure coincidence, and that at best irradiation may aggravate an already existing coronary lesion.

Summary

In 40 patients suffering from oesophageal carcinoma ECG's were made before and immediately after irradiation of a tumor dose of 3 000 and 6,000 R. In 11 patients 14 ECG's also were made between 3 and 13 months after the end of the irradiation. It was impossible to find any deterioration. Post mortem examinations made in five patients showed no changes in the heart muscle which could be attributed to irradiation. It therefore is thought by the author that irradiation of tumors in or on the chest is far less damaging to the heart than other publications may suggest.

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after irradiation of an unidentified tumor in or on the chest in two female patients who had never before suffered from angina.

Jones and Wedgewood (2) supposed that changes in the ECG in their patients with carcinoma of the breast were only likely to occur if signs of coronary disease were already present before the irradiation.

Methods

To estimate the effects of X ray therapy of intrathoracic tumors on the hearts of our patients we made ECG s before, during and after treatment. We selected only patients suffering from oesophageal carcinoma. As is known this tumor has three sites of predilection. The higher situated tumors may be irradiated without affecting the heart and were therefore intended as a possible control group The heart receives progressively more X rays the lower the tumor is situated at the bifurcation or just above the cardia.

The irradiation (Dr R. J Gielen) of our patients took place according to the following scheme. At the front and the back of the chest three fields each were drawn diagonally opposed to each other Their width was 6 cm, their length depending on the size of the tumor Every day two oppositely situated fields were irradiated with a skin dose of 200 R each, while the fields 1 2 3, always rotated in the same order The total number of irradiations was dependent of the thickness of the patient and was finished after the tumor had received a total of 6,000 R.

High located oesophageal tumors received X-ray treatment from 4 such fields of which two were situated at the anterior and two at the posterior side of the thorax.

In some cases low situated tumors have been irradiated from two such fields only one in the centre front, one in the centre back of the thorax.

In the period 1957—1960 we made ECG s of 40 patients. The carcinoma was situated high in 6 (group I) at the bifurcation in 21 (group II) and low in the oesophagus in 13 patients (group III)

Results

In the first group all patients had an essentially normal ECG according to their age. This did not change after irradiation of 3 000 R and in the 5 patients in which we were able to make an ECG after 6 000 R it had not changed either

In the second group before treatment the ECG was normal for their age in 15 patients and pathological in 6 of them. In all patients with a normal ECG no changes were observed after 3 000 R and 6 000 R. Of our 6 patients with a definite pathological ECG (two R. B B B. and four with signs of left strain) there were no changes in 4 In the two other cases with left strain these changes became less marked probably due to rest and antihypertensive therapy

In the third group with the low situated carcinoma in which the heart received the biggest quantity of X rays before treatment the ECG was normal in ten and pathological in three patients. No changes were observed during radiation.

As Pearson (3) described two cases of coronary infarction about 6 months after irradiation we made 14 ECG s in 11 patients between 3 and 13 months after the end of the therapy None of these showed any changes compared with the former ECG s.

Post mortem examinations were made in 4 patients of group II and in one of group III According to our pathologist (Dr R Eibergen) no changes could be found in the heart muscle which could be attributed to irradiation

Discussion

Although some authors reported changes in the ECG which were attributed to damage to the coronary vessels by X ray therapy to tumors in or on the thorax,

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Cyclic Neutropenia

Report on Three Cases

By

Aage Videbæk

Cyclic neutropenia, syn. cyclic or periodic agranulocytosis, manifests itself as regular episodes of symptomatic neutropenia, usually persisting throughout life. However the period of possible complete agranulocytosis is followed by a rapidly increasing neutrocyte count, and all symptoms may disappear until the next episode of neutropenia sets in, usually 21 days after the commencement of the preceding episode.

The disease is rare. Page and Good, in 1957 (24) could not collect more than 23 sufficiently substantiated cases from the literature. To these may be added their own case and at least 17 additional cases (1 4 5 6, 8, 9 10 13, 14 18 19 22, 25 27). The disease has been encountered in a number of different countries, and there has been no significant geographic distribution. One of the reported cases occurred in a Negro (9) while all the other patients have been Caucasians. In Scandinavia 6 cases have been observed, including one of the present author patients (1 15a, 23, 25 30). There is perhaps a slight female preponderance (about 3:2). In the great majority of cases the disease has had its start in infancy in 9 cases it apparently did not begin until adult life but only in 3 cases after the age of 50 (18, 21 28) including a 76-year-old patient (21).

Since evidently the disease is rare 3 cases will be reported two of the patients being father and son.

Case histories

Case 1 A 29-year-old clerk with a history from the 2nd year of life, of episodes manifesting themselves as fever (maximum 39° C) of few days duration, later also periodical enlargement of the cervical lymph nodes, tenderness of the gums, sometimes small white ulcers on the oral mucosa, dysphagia, and sometimes conjunctivitis. From the age of 4 or 5 months obstinate furunculosis which was constantly present for 18 months. Since that time few furuncles, but several sore throats.

Admitted to the University Hospital, Paediatric Department, 5 times from 11 to 18 years of age, where cyclic neutropenia with simultaneous monocytosis was diagnosed (23) as early as 1943, when the patient was 11. The spleen was not enlarged.

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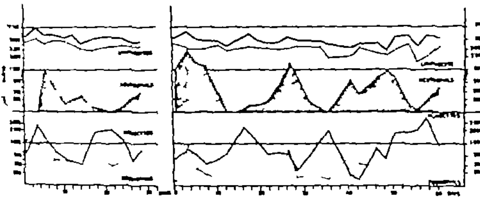


Fig. 2. Case 2. Pronounced cyclic variation in the neutrophil granulocyte and monocyte counts during 2 periods of one and of two months.

During the second year of lif once every month fever up to approx. 38 C, on two occasions also paronychia. When the boy was first seen here such an episode of 2—4 days had occurred a week previously. On the inside of the lower lip there was a bleb and tw ulcers of a few mm in diameter. The parents refused to have the boy admitted. Thanks to collaboration with the local central laboratory (Esbjerg) ambulatory white cell and differential counts were performed every other day for long time. On the basis of the findings fig. 2 shows the results from a 29-day period and another period of 2 months. At intervals of exactly 21 days, the neutrophil count dropped to 0 or very lose to 0 where it remained for 4—8 days in order to increase again up to maximum value 1,000, 4,000 1,300 and 1,000 respectively in the four 21-day periods. The monocyte count varied regularly reaching up to 2,000 per l blood when the white cell count was at minimum, and the monocyte count was low when the neutrophil count was at maximum. The neutrophil count did not at any time rise to normal values. Treatment with penicillin was started 300,000 I. U. every 12th hour during the subsequent neutropenic periods there was no elevation of temperature and the boy appetite as well as general condition were satisfactory. During one period, however he got scratch on the lip which became infected, and during another period he had balanoposthitis.

The father of the child (case 1) has healthy sister and his parents are not blood relations. Among 27 close relatives ther was no instance of periodic disease (fig 3). Although the neutropenia recurred in both cases at intervals of exactly 3 weeks, its onset was not simultaneous in the two patients.

Case 3. A male aged 18 years. From infancy attacks of tonsitis and conjunctivitis ac companied by fever. These attacks would last for about week and came on every 3 weeks. In between he was in good health. About 11 years previously attacks occurred about every tw weeks and were associated with tenderness of the gums, headache, ar ticular and muscular pain. N serious local or generalized infections,but several sore throats every year

H was admitted at the age of 18 because of gross dental caries, and at the Dental Clinic of the University Hospital (Professor J. J. Pindborg) the diagnosis was advanced, progressive marginal paradentosis with severe bony destruction and large masses of calculus.

Cervical axillary and inguinal lymph nodes slightly enlarged, but non-tender. Spleen not palpable. Hb. 13.2 g/100 ml, W. B. C. 3 100 with 4 % segm. neutrophils, % rod shapes, 2 metamyelocytes, 27 % eosinophils, 1 % basophils, 39 lymphocytes, and 20 % monocytes. E. S. R. 25 mm. No demonstrable leukocyte agglutinins (Dr Gert Jensen). Paper electrophoresis Slightly elevated -globulin (27.2 %). The patient ery quickly discharged himself.

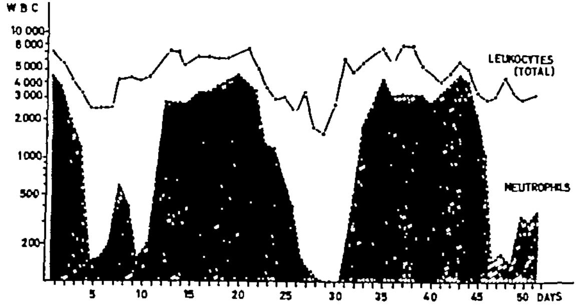


Fig 1 Case 1 Pronounced cyclic variation in the neutrophil granulocyte and leukocyte counts during a period of 51 days.

In 1952 when the patient was 20 the episodes of cyclic neutropenia were still occurring at 21-day intervals. They were associated with fever tenderness of the gums, and oral aphthous ulcers.

Fig 1 shows a neutrophil count between 0 and 500 through approx. one week. Towards the end of the agranulocytic phase the sternal marrow showed a marked shift to the left in the myeloid series. Myeloblasts 0 promyelocytes 1 % n. myelocytes 27 % n. metamyelocytes 17 n. rod shapes 17 % n. segm. 5 immature eosinophils 4 mature eosinophils 5 lymphocytes 17 plasma cells 4 smudge 3 % there were 17 normoblasts per 100 of the named cells. Corticotrophin, in a daily dosage of 20 units for 6 days, was tried partly in a period of agranulocytosis and partly immediately before the onset of such a period. However this did not affect the cyclic variation in the leukocyte count, but the fever was not so high and the general condition better

In 1958 (then aged 25) he was still disabled for 2 weeks of each month by these symptoms, especially from the oral cavity There was still no splenomegaly E. S R. 4—6 mm Hb. 15 1 g/100 ml, platelet count normal. During the stay in the Department, for 11 days, the neutrophil granulocyte count ranged from 700 to 1,200 total protein 8.15—8.98 g/100 ml albumin 4 16 α_2-globulin 0 68 β-globulin 0.85, β_1-globulin 0.68, γ-globulin 1.53 g/100 ml.

Since 1958 he has been on permanent oral penicillin medication, originally 300,000 I U twice daily but during the past year only 3 times weekly During these 3 years he has been confined to bed only 2 or 3 times with "influenza and has had stomatitis only once.

At out patient follow-up in August 1961 he had only one ulcer on the buccal mucosa, palmar erythema, and one spider naevus. The spleen was not palpable below the costal border and there was no hepatomegaly Hb. 14 7 g/100 ml, E. S. R. 3 mm, W B. C. 2 400 with 23 segmental neutrophils, 4 monocytes, and 69 lymphocytes. Platelets 139 000. Serum bilirubin 1 1 mg/100 ml, prothrombin time 74 total protein 8.3 % paper electrophoresis normal.

Case 2. (Haematological Out-patient Department, August 1961) A boy aged 2 years (son of case 1) born normally at home as No. 1 of a family of one. In good health until the age of 10 months when his first tooth erupted. Since that time recurrent sore throats and stomatitis associated with enlargement and tenderness of the lymph nodes at the angle of the jaw

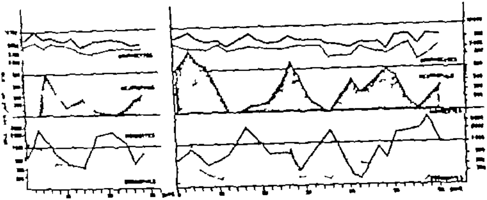


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H was admitted at the age of 18 because of gross dental caries, and at the Dental Clinic of the University Hospital (Professor J. J. Pindborg) the diagnosis was advanced, progressive marginal paradentosis with severe bony destruction and large masses of calculus.

Cervical, axillary and inguinal lymph nodes slightly enlarged, but non-tender Spleen not palpable. Hb. 13.2 g/100 ml, W. B. C. 3 100 with 4 segm neutrophils, 7 rod shapes, 2 metamyelocytes, 27 % eosinophils, 1 basophils, 39 lymphocytes, and 20 % monocytes. E. S. R. 25 mm. No demonstrable leukocyte agglutinins (Dr Gert Jensen) Paper electrophoresis Slightly elevated γ-globulin (27.2 %) The patient ery quickly discharged himself.

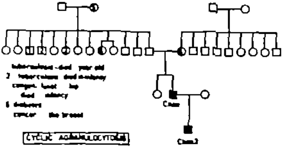


Fig 3 Case 1 and 2 Pedigree showing cyclic neutropenia in a father and son No other cases of periodic disease in the family

Discussion

A priori it seems likely that a difference exists between the apparently congenital cases and those which manifest themselves in adult life. This assumption is also supported by the fact that cases having their start in infancy appear to persist throughout life, in some cases definitely for 30—40 years (the present author's oldest patient and 8a, 10a, 11 and 29) while a number of the cases observed at an older age have been of a fairly short duration have not shown such a regular cycle (1 10) or else the cyclic neutropenia may have been secondary to other diseases, such as lymphosarcoma (28) Brill-Symmer's disease (22) or agammaglobulinaemia (12)

Family data are sparse and naturally of limited value in the absence of systematic family studies. But just as chronic neutropenia is known to have run in families (15 16 17) the familial occurrence of cyclic neutropenia has been observed frequently enough to attribute a certain significance to familial, perhaps genetic factors. For instance cyclic neutropenia has been found in a 16-year-old female who was observed for one year Her baby died at the age of a few months from agranulocytosis which judging by the report was also cyclic (4) Out of 2 brothers with congenital agammaglobulinaemia one had cyclic neutropenia while the other one suffered for 2 months from constant neutropenia which then ceased (12) Lastly cyclic neutropenia has been described (14) in a father who had suffered from the disease for 16 years, and his daughter whose symptoms started at the age of 3 months, but not as a typical cyclic neutropenia until the age of 10 months. To these cases may now be added the present two cases of cyclic neutropenia in a father and his one year-old son (cases 1 and 2) In these last two cases a common exogenous cause appears to be unlikely as the variations in the neutrophil counts were not synchronous.

Haematological course of the cycle

The length of this cycle is in most cases 20—22 days, and the attacks recur with great precision. A duration of 14 days has been reported (1 14 and the present case 3) and up to 28 days (2 2a, 10a) in one case up to 45 days (15 a) but in that event the rhythm is not so regular For 2—4 days the neutrophil count is extremely low sometimes 0 whereupon it rapidly increases, in many cases only up to subnormal values, while in some cases normal values are attained Repeated smears of bone marrow at various stages of the cycle have shown that concurrently with the blood neutropenia the bone marrow shows not only a very low neutrocyte count but also very few myeloblasts, promyelocytes, and myelocytes. The myeloblast and a little later the myelocyte count rises considerably a few days before the blood neutrophils. This finding indicates that the blood neutropenia is due to a reduced granulocyte production not to an increased destruction of leukocytes in the blood stream. And indeed no one has definitely demonstrated leukocyte agglutinins which

might be held responsible for such peripheral neutropenia (cf. 4 14 24 and the present case 3) The total white cell count often accompanies the fluctuation in the neutrophil count. Since as in the youngest patient of the present series, there is often marked monocytosis during the agranulocytic phase the aggregate result may be that the total white cell count remains on the whole fairly unchanged despite marked fluctuations in the number of neutrophilic granulocytes. There does not appear to be a regular alteration in the eosinophil count. Platelet counts have not infrequently been reported to be high (in one case low (29)) The repeated infections are presumably responsible for the fact that a number of patients with elevated γ-globulin (1 4 24) may have a slightly increased number of plasma cells in the bone marrow in some cases as early as the first months of life (4) The combination with agammaglobulinaemia appears more peculiar as it has been found that out of 8 children with agammaglobulinaemia not less than one had persistent, two transitory and one cyclic neutropenia (12) In most cases the spleen and liver have not been palpable, but occasionally the patients have shown slight splenomegaly In cases where splenectomy has been performed the weight of the spleen has often been stated to be about 300 g, maximum 520 g (21) The removed spleens have not shown any particular histological change (except in the few cases with malignant systemic disease) In one case (21) the sinus endothelium was said to be hypertrophic.

The E. S. R. may fluctuate in the individual cases between high values corresponding to the neutropenia, and normal values when the neutrophil count is normal (6, 9 26)

Clinical course of the cycle

In the great majority of cases the symptoms have been restricted to 4—10 days around the neutropenic phase. The most constant signs are those from the oral cavity and pharynx in the form of aphthous ulcers, necroses possibly pharyngeal erythema, tonsillitis, gingival swelling and tenderness, possibly gingival bleeding and enlargement of the regional lymph nodes. The term periadenitis necrotica mucosae recurrens has occasionally been used (4 5 13) Abdominal pain and diarrhoea are rare, while fever and articular pain are not uncommon. One case exhibited conjunctivitis, another recurrent vulval ulcerations (2) and infants, in particular have shown recurrent furunculosis and cutaneous papules. More uncommonly there have been serious, alarming infections, such as pericolitis with abscesses pulmonary abscess, septicaemia, and even valvular endocarditis. Death may occur if the necroses are complicated by intestinal perforation (4 9 27) or intestinal haemorrhage (26) Pregnancy has in some cases alleviated and in others aggravated the symptoms and signs. In the great majority of cases the infections are mild. This is in keeping with the fact that many patients have managed fairly well for many years. While they may be fairly debilitated and bedridden during the few days of maximum neutropenia, they often feel perfectly well during the 2 or 3 weeks elapsing until the onset of the next neutropenic phase

Treatment

So far it has proved impossible to break the cyclic variations in the neutrophil count by ACTH or cortisone (6, 19 21 22) prednisone (1 24) antibiotics or sulphonamides (23) vaccine (24) or

splenectomy However splenectomy — performed in 14 cases — has appreciably reduced the symptoms, especially if there had been splenomegaly and the patient was over 20—30 years of age (8 9) Warnings have been given against the prophylactic use of antibiotics, despite clinical improvement, as this might give rise to infection with penicillin resistant bacilli (24) However oral treatment with penicillin completely altered the condition in one of the present patients, and in the second case fever did not occur after penicillin was instituted. *It also seems reasonable, especially during* phases of neutropenia, to keep the patients isolated from persons with infections.

Summary and conclusions

Three cases of chronic cyclic neutropenia are reported Two occurred in a father and his son. The third patient had extremely advanced marginal paradentosis with bony destruction.

Forty cases are on record The pathogenesis manifestations, course, and treatment of this peculiar periodical disease are briefly discussed

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Serum Ornithine Carbamoyl Transferase Activity in Man

A Highly Specific Test of Liver and Biliary Tract Involvement Observation on 695 Patients

By

Hans Reichard

As has been reported earlier in man ornithine carbamoyl transferase (1) occurs mainly in the liver (2, 3) of other organs, only the small intestine contains significant amounts (3). In normal subjects the serum ornithine carbamoyl transferase activity (S-OCT) is very low (3). Findings from previous studies suggest that an increase in the activity of this enzyme is specific for liver damage (4—10) and the object of the present investigation was to establish whether this is in fact the case, and to ascertain whether S-OCT determination is a more sensitive test than others usually employed.

Material

Observations of S-OCT were made on 695 subjects, 560 of whom were patients at the Departments of Medicine and Rheumatology of the Karolinska Hospital, 61 at the Departments of Medicine and Surgery of St. Erik's Hospital, 42 at Långbro Mental Hospital and 32 at the Stockholm Hospital for Contagious Diseases. Apart from less than 20 patients whose records were very incomplete all patients were included where two or more samples had been taken for determination of S-OCT during the period 1st June to 31st December 1956, or where at least one sample had been obtained between 1st January 1957 and 30th September 1961.

The patient records were examined with special attention to data on previous liver and biliary tract diseases, consumption of alcoholic beverages, circulatory insufficiency, malignant diseases and results of liver tests. The record data on these points varied greatly from one patient to another. In 18 cases with elevated S-OCT and uncertain diagnosis follow-up examinations were performed.

Patients who had had more than one disease were classified according to the one that was most frequently accompanied by an increase in S-OCT. In the case of multiple diseases that are usually not associated with an increase in the level of this enzyme the patient was classed under the main disease for which he had been admitted to hospital.

The specimens of venous blood taken for the S-OCT determinations were usually drawn in the morning. After standing at room temperature for 1—3 hours they were centrifuged and the serum was stored at — 20° C. Under these conditions the S-OCT was stable over periods of several months (11). The analyses were performed as a rule within one week, and always within 3 months.

Submitted for publication, June 26, 1962.

Methods

The serum ornithine carbamoyl transferase activity was determined by incubation of serum with citrulline carbamoyl ^{14}C in arsenate buffer by the method of Reichard and Reichard (11) The results were expressed in micromoles of $^{14}CO_2$ liberated by 0.5 ml of serum *under standard conditions.* On the basis of normal values for persons above 25 years of age, the S-OCT values obtained were classed as normal ($\lesssim$ 0 060 μM) probably elevated (0 061—0 100 μM) and significantly elevated (> 0 100 μM) The upper normal limit calculated with 95 % confidence for 95 % of the distribution was 0.060 μM. The corresponding value for 99 of the distribution was 0.100 μM. For persons of 25 years or less the upper normal limit for 95 % of the distribution was 0 030 μM (12)

The other tests and determinations were as follows

Determination	*Method*	*Normal range*
S-GOT[1]	Reitman & Frankel (13)	$\lesssim$ 40 units (14 — 16)
S-GPT[2]	Reitman & Frankel (13)	$\lesssim$ 35 units (14)
S-AP[3]	Kaplan & Narahara (modified) (17)	$\lesssim$ 7 units[*]
Icteric index	Meulengracht (18)	$\lesssim$ 8 units[*]
Thymol turbidity	Maclagan (19)	$\lesssim$ 4 units (19)
Neoprontosil test	Siede & Schneider (20)	$\lesssim$ 105 mg in 24 hr urine (21)
Bromsulphalein test	5 mg/kg body wt	$\lesssim$ 6 % retention after 45 min. (22)

S-GOT = Serum glutamic oxalacetic transaminase activity
S-GPT = Serum glutamic pyruvic transaminase activity
S-AP = Serum alkaline phosphatase activity

* Normal values from record cards at the Department of Biochemistry Karolinska Sjukhuset.

Results

More than 3 000 sera were assayed from the 695 patients examined. The results relating to the 695 patients are given in table I and fig 1 which are based on the highest of the S-OCT values recorded for each patient. Serial S-OCT determinations were made for most of the cases of hepatitis liver cirrhosis, alcoholism and acute enteritis, and in many of the cases of biliary stasis, circulatory failure, myocardial disease and rheumatoid arthritis.

Infectious hepatitis

All 23 cases of epidemic and homologous serum hepatitis and 2 of mononucleous hepatitis had elevated S-OCT The highest level was usually that recorded on admission when the patient had been jaundiced for one or more days (fig 2) The values then fell rapidly often to normal prior to normalization of the icteric index (fig 3) The relationship between the degree of jaundice, S-OCT S-GOT and S-GPT in one illustrative case is shown in fig 3 in which the parallel course of the measured enzyme activities is also evident.

The S-OCT remained elevated in 3 patients where there was not complete clinical recovery Fig 4 shows the course for one of them

Cirrhosis of the liver

In 34 out of 48 cases of cirrhosis there was microscopic confirmation of the disease. The remaining 14 cases satisfied at least five of the following eight criteria 1 history of alcoholism 2 history of infectious hepatitis with recurrent jaundice, 3 jaundice, 4 enlarged spleen

Table I. Serum ornithine carbamoyl transferase activity in 695 cases of various diseases

Diagnosis	No. of cases	S-OCT level		
		Normal	Probably elevated	Significantly elevated
Infectious hepatitis	25	—	—	25
Liver cirrhosis	48	8	2	38
Other liver diseases	10	2	2	6
Hyperbilirubinaemia	6	6	—	—
Gall stones				
a) Jaundice	15	—	1	14
b) Acute cholecystitis	9	—	1	8
c) Silent gall stones	10	10	—	—
Cancer				
a) Jaundice	13	2	3	8
b) Liver metastases	16	6	3	7
c) No liver metastases	17	17	—	—
d) Systemic malignant diseases	15	9	3	1
Alcoholism				
a) With delirium tremens	33	2	6	27
b) Without delirium	24	4	6	14
Circulatory failure				
a) Tachycardia and fall in blood pressure	13	—	3	10
b) Congestive heart failure	22	8	2	12
Myocardial disease	47	36	4	7
Rheumatoid arthritis	67	58	5	4
Systemic lupus erythematosus and allied collagen conditions	30	19	2 + 2	7
Other diseases with joint and muscle symptoms	141	132	5	4
Gastric and duodenal ulcer and gastritis	15	14	1	—
Diseases of the small intestine	16	10	2 + 2	2
Ulcerative colitis	11	10	—	1
Blood diseases (not malignant)	16	16	—	—
Miscellaneous	76	66	3 + 1	6

S-OCT is expressed in μM CO_2 in 0.5 ml serum. Normal, ≲ 0.060, probably elevated, 0.061—0.100 and significantly elevated > 0.100 μM.

Comprises 16 cases of systemic lupus erythematosus, 4 of obscure collagenoses, 3 each of dermatomyositis and scleroderma and 2 cases each of polymyalgia rheumatica and polyarteritis nodosa.

Patients of 25 years or less with S-OCT activity of 0.05—0.06 μM that is, probably elevated for their age.

and/or oesophageal varices, 5 ascites, 6 spontaneous lowered prothrombin index, 7 elevated gammaglobulin in serum (> 1.6 g/100 ml) and lowered serum albumin (< 3.6 g/100 ml) determined by paper electrophoresis, 8. reduced capacity of the liver for eliminating injected bromsulphalein or neoprontosil.

Forty out of the 48 patients had elevated S-OCT. The highest values were recorded for three cases of decompensated cirrhosis (jaundice, ascites, intestinal bleeding or coma). Among the other 45 cases no difference in S-OCT was observed between decompensated and compensated cirrhosis.

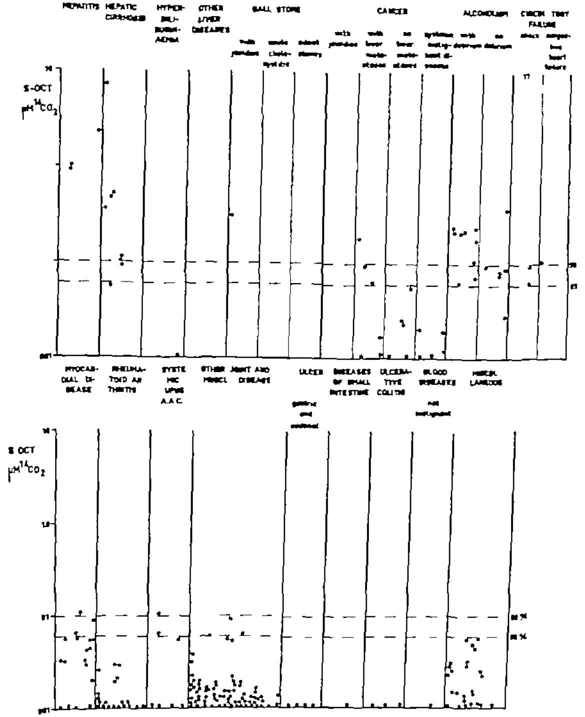


Fig 1 S-OCT in 693 cases of various diseases. The two broken lines indicate the upper normal limit of 95 and 99 % of the healthy subjects of both sexes over 25 years of age. The corresponding upper normal limits for younger subjects are somewhat lower S-OCT on logarithmic scale. (denotes subjects not more than 25 years of age and with S-OCT f 0.030 — 0.060 μM.)

Normal S-OCT values were obtained for 8 patients for 6 of whom the diagnosis was confirmed by biopsy In 5 of these 8 the cirrhosis was decompensated in type. The cause of the disease in 5 cases was alcoholism and in 2 infectious hepatitis

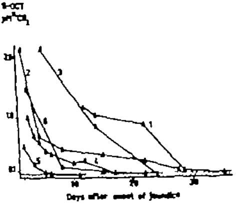


Fig. 2. S-OCT in 6 cases of infectious hepatitis. The shaded area denotes the normal S-OCT

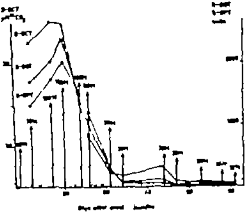


Fig. 3. S-OCT S-GOT and S-GPT for 74-year old woman with homologous serum hepatitis from whom the first samples were taken before the maximum serum level had been reached. M = icteric index. The shaded area denotes the normal enzyme activities.

in one the cause was unknown. One of the cases of chronic hepatitis received continuous steroid therapy

In table II the results of the S-OCT determinations are compared with those for S-GOT S-GPT S-AP icteric index, neoprontosil and bromsulphalein tests on the 40 cases of cirrhosis from the Karolinska Hospital. The comparisons relate to the first samples taken on admission to hospital. As the table shows, S-OCT was more often elevated than S-GOT S-GPT or S-AP The difference was significant only for S-GPT and S-AP The neoprontosil and bromsulphalein tests gave pathologic values insignificantly more often than the S-OCT determinations.

Liver diseases other than infectious hepatitis and cirrhosis

This group consisted of 10 cases of various hepatic conditions other than definite infectious hepatitis or cirrhosis.

The S-OCT was normal for the one case of amyloidosis of the liver and one of liver abscesses. It was probably elevated for another patient with liver abscess and one of multiple diseases, poor



Fig. 4. S-OCT and S-GOT for 23-year-old man with epidemic hepatitis who had not recovered 5 months after the onset. B = serum bilirubin. The shaded area denotes the normal S-OCT level.

general condition and slight, obscure jaundice, not due to haemolytic anaemia. Significantly elevated values were recorded for 6 patients 2 with liver damage probably due to drugs — one each to β-phenyl isopropyl hydrazine (Catran®) and chloropropamide (Diabinese®) — 2 with probable hepatitis but no jaundice, one with aetiologically obscure diarrhoea and markedly pathologic findings on liver puncture, and one with severe fattening of the liver of several years standing

Table II S-OCT compared with S-GOT S-GPT S-AP icteric index neoprontosil and bromsulphalein tests for 40 cases of liver cirrhosis. A statistical evaluation of the number of pathologic results of the various tests each compared with S-OCT

	No. of cases	S-OCT normal		S-OCT pathologic		Comparison between B and C	Level of significance
		Other test		Other test			
		Normal A	Pathologic B	Normal C	Pathologic D		
S-GOT	32	0	*2*	*6*	22	B < C	P > 0,05
S-GPT	29	2	*0*	*14*	13	B < C	P < 0,001
S-AP	39	4	*2*	*14*	19	B < C	P < 0,01
Icteric index	38	3	*3*	*8*	24	B < C	P > 0,05
Neoprontosil test	20	0	*4*	*0*	16	B > C	P > 0,05
Bromsulphalein test	12	0	*1*	*2*	9	B < C	P > 0,05

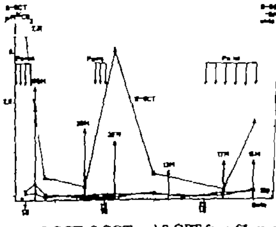


Fig 5 S-OCT S-GOT and S-GPT for a 63-year old woman with intermittent biliary stasis du to choledochal stone. M = icteric index. Note the close connection between pains and elevation in S-OCT the transaminases were not affected to the same extent. The shaded area denotes the normal enzyme activities.

Hyperbilirubinaemia

Normal S-OCT values were recorded in 2 cases of Dubin Johnson syndrome (verified by biopsy) and 4 of post hepatic or congenital non-haemolytic hyper bilirubinaemia, for 3 of whom liver punctures were performed.

Gall stones

a) *Jaundice* This group consisted of 6 cases of choledochal stone confirmed at operation, 5 of jaundice probably due to choledochal stone 3 of cholecystitis and jaundice and of one of cholangitis after operation for cholecystitis. All 15 patients had elevated S-OCT Extremely high values were observed only where the jaundice was of shorter duration than one week or where the obstruction was of an intermittent type, as in the case illustrated in fig 5 S-OCT in this case increased to 100 times the normal value within 24 hours of the onset of biliary stasis and then fell when the occlusion was released The transaminases reacted to a lesser degree. It is obvious that there was an association between the repeated pains due to stone and the rise in S-OCT

b) *Acute cholelithiasis* (no jaundice) All 9 patients with clinical symptoms of gall stones where the diagnosis was confirmed at operation or radiographically showed increased S-OCT levels in connection with acute attacks of pain The enzyme activities in serum appeared to reach their peak not more than a few hours

ter the onset of the pains. In one case e S-OCT was almost normal 2 days fter the attack of stones.

c) *Silent gall stones* The S-OCT was ithin normal limits for 10 cases of gall ones verified at operation or by radiography where the blood samples were aken during a symptom-free period.

CANCER

a) *Jaundice* Most of the 13 patients in his group were jaundiced owing to iliary obstruction of the biliary tree by the tumor In one or two cases however hepatocellular failure might have contributed to the jaundice. All 13 patients had elevated S-AP

Elevated S-OCT was ecorded in 11 cases of this group The 2 cases with normal values had jaundice of more than one month s duration and 3 with probably elevated levels had also had jaundice for a longer period. Very high values were found chiefly but not exclusively in cases with jaundice of short duration.

b) *Liver metastases* (no jaundice) Out of 16 non-jaundiced cases of cancer of the liver 6 had normal and 10 elevated S-OCT There was no apparent parallel between the extent of the metastases and the S-OCT level.

Normal levels of S-GOT S-GPT and S-AP were recorded in 6 out of 6 6 out of 7 and 6 out of 13 cases, respectively (table III)

c) *No liver metastases* Normal S-OCT was recorded in all 17 cases of cancer 4 of them with no metastases at all and 13 with metastases in the skeleton and/or soft tissue, but not in liver

d) *Malignant systemic diseases*. This group of 13 patients consisted of 3 cases of malignant lymphogranulomatosis, 3 of myeloma, 5 of acute myelotic leukaemia, one of chronic lymphatic leukaemia and

Table III S-OCT compared with S-GOT S-GPT and S-AP for anicteric cases of primary or secondary carcinoma of the liver

	No. of cases	S-OCT normal		S-OCT pathologic	
		Other test		Other test	
		Normal	Pathologic	Normal	Pathologic
S-GOT	6	2	–	4	–
S-GPT	7	2		4	1
S-AP	13	3	1	3	6

one of reticulosarcoma. Nine had normal S-OCT in 3 the level was probably elevated and in one significantly so. This last patient had acute myelosis and at autopsy the liver was found to be enlarged and massively infiltrated with immature cells.

ALCOHOLISM

Out of 35 cases of classic delirium tremens and 24 of alcoholism without delirium, 52 had elevated S-OCT and 6 normal levels. No difference in S-OCT was evident between these two groups. In both, S-OCT usually reached its peak during the first days after admission and then decreased over some 10 days (fig 6) There was, however an occasional increase in S-OCT 3—5 days after admission.

Normal S-OCT levels were recorded for 2 patients from whom blood samples were taken 2 and 4 days after the onset of delirium, for 2 patients without delirium who had consumed alcohol on the days before the specimens were taken, and for 2 patients without delirium who had abstained more than one week before the specimens were taken.

To compare S-OCT and S-GOT for alcoholics, a closer examination was made

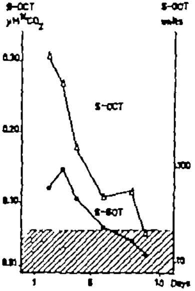


Fig 6. S-OCT and S-GOT for a 48-year-old alcoholic with moderately severe alcoholic delirium, which subsided on the fifth day The shaded area denotes the normal enzyme activities.

of the group of cases from Långbro Mental Hospital that had been subjected to continuous and consistent examination (23) Determinations of S-OCT and S-GOT were made on 114 samples from 32 delirants and 10 alcoholics without delirium 18 samples showed elevated S-OCT and normal S-GOT 15 showed normal S-OCT and elevated S-GOT (table IV) the difference, however is not significant. Nor was there any difference for this comparison when the series was grouped into alcoholics with and without delirium. On the other hand when the series was divided with respect to the time after admission a difference was evident. Of 23 alcoholics followed after the sixth day in hospital 10 had increased S-OCT combined with normal S-GOT whereas none had normal S-OCT *with pathologically* increased S-GOT This difference is significant (table V) but not so the difference for the corresponding comparison for the first 6 days in hospital.

Table IV S-OCT compared with S-GOT for 114 tests on a group of 42 alcoholics, 32 of whom had delirium. The values are taken from table I of Lundquist, Nettelbladt and Reichard (23)

S-OCT normal		S-OCT pathologic		Comparison between B and C	Level of significance
S-GOT		S-GOT			
Normal A	Pathologic B	Normal C	Pathologic D		
13	*15*	*18*	68	B < C	P > 0.05

Table V S-OCT compared with S-GOT for 23 samples from 23 alcoholics. The samples were taken at least 7 days after onset of delirium or in cases without delirium, at least 7 days after admission. The values are taken from table I of Lundquist, Nettelbladt and Reichard (23)

S-OCT normal		S-OCT pathologic		Comparison between B and C	Level of significance
S-GOT		S-GOT			
Normal A	Pathologic B	Normal C	Pathologic D		
4	*0*	*10*	9	B < C	P < 0.01

As has been shown in an earlier paper (23) in the series from the Långbro Mental Hospital there was no parallel between, on the one hand, elevated S-OCT and on the other increased bilirubin content in serum or pathologic thymol turbidity

CIRCULATORY FAILURE

a) *Increase in pulse rate and fall in blood pressure* This group of 13 patients with a great increase in pulse rate (exceeding 150 beats/min) and fall in systolic blood pressure exceeding 60 mm Hg was composed of 8 cases of cardiac infarction, and one each of multiple pulmonary

embolism, aortic stenosis probably with acute forward failure of the heart, shock due to severe gastric haemorrhage and one with shock at the final stage of inoperable lung cancer This last patient showed a drop in blood pressure from 240/120 to 130/100 but no marked increase in pulse rate. The thirteenth patient was one of heat stroke with extreme tachycardia and apparently low blood pressure he was included in this group, but with reservation.

All the patients had elevated S-OCT levels but the range was wide (fig 1) Three out of the 4 highest values were recorded for patients with irreversible shock. In the cases of less marked increases, the association of the duration of the fall in blood pressure and increase in S-OCT was not so obvious. As a rule the rise in S-OCT was not noted until 2 days after the fall in blood pressure (fig 7).

b) *Congestive heart failure.* To this group were assigned patients with circulatory failure in the form of lung oedema, pleural transudate, ascites or marked oedema of the legs, and those who responded to diuretic therapy with a rapid and marked loss of weight. In none of these cases were there obvious signs of shock (elevated pulse rate and drop in blood pressure). Out of the 22 cases of congestive heart failure, 8 had normal S-OCT 2 probably and 12 significantly elevated S-OCT Normal levels were recorded mainly in cases of lung oedema and stationary moderate degrees of incompensation.

For the 14 patients where the S-GOT was also determined the S-OCT was elevated in 8 nd the S-GOT in 6. In the 1 cases where the S-GPT was assayed the S-OCT was levated in 6 and the S-GPT in 4

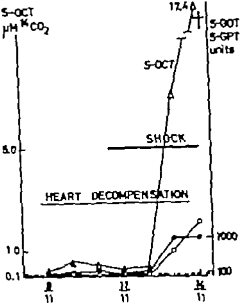


Fig. 7 S-OCT S-GOT and S-GPT for a 56-year old man with valvular heart disease and moderate heart decompensation at admission. On 10th November the blood pressure fell and the patient lost consciousness. The shock was irreversible and there was an increase in the RN level. Slight jaundice was noted on the day before death. The shaded area denotes normal enzyme activities.

Myocardial disease

This group consisted of 16 cases of acute myocardial infarction and 31 of stenocardic disorders. In none of them were obvious signs of heart decompensation or low blood pressure noted. Out of the total of 47 patients, 36 had normal S-OCT and 4 probably elevated levels. The 7 with significantly elevated S-OCT were the following

1 A 74-year-old man, who had earlier undergone cholecystectomy S-OCT 5 days after myocardial infarction was 0.110 μM and S-GOT and S-GPT were both elevated.

2. A woman of 54 years with myocardial infarction. An S-OCT of 0.070 μM on admission rose to 0.220 in 5 days and then returned to normal at 0.046 μM. At the same time S-GPT also rose to slightly pathologic levels.

3 A man of 60 years with myocardial infarction. S-OCT was 0.330 μM on the first day and returned to normal in 3 days.

4 A man of 54 years with myocardial infarction but otherwise in good general condition. Six days after the infarction S-OCT rose to 0 160 μM, the S-GOT to 110 units and S-GPT to 83 One week later all the serum enzyme levels were normal.

5 A man of 67 years with myocardial infarction 3 months previously admitted because of frequent attacks of angina pectoris and dyspnoea. The general condition was bad and slight oedema of the legs was noted During the first days in hospital S-OCT rose from 0 032 to 0 115 μM and S-GPT from 20 to 33 units.

6. A woman of 66 years with severe angina pectoris and inexplicable S-OCT of 0 129 μM At a follow up 4 years later liver tests and S-OCT were normal.

7 A man aged 39 years, who had previously undergone cholecystectomy and who had a second infarction 16 days previously with no signs of circulatory failure. S-OCT was 0.210 μM.

RHEUMATOID ARTHRITIS[1]

Out of 67 cases who satisfied the diagnostic criteria for rheumatoid arthritis according to Ropes et al. (25) 58 had normal S-OCT 5 probably elevated and 4 significantly elevated levels. The mean S-OCT (0 023 μM) in this group did not differ significantly from the mean (0 019 μM) for a normal material (12) All elevated values were observed in women, and then mainly in early cases. There was no evident causal connection between the therapy (chloroquine and phenylbutazone) and the S-OCT level, which moreover was apparently also unrelated to the other liver tests (24)

SYSTEMIC LUPUS ERYTHEMATOSUS AND ALLIED CONDITIONS

The mean S-OCT (0 037 μM) and the standard deviation for this fairly heterogenous group of 30 cases were much higher than for a normal material (12) (The differences were highly significant $P < 0 001$)

a) *Systemic lupus erythematosus* Out of the 16 cases with this disease, 12 had normal one probably and 3 significantly elevated S-OCT The 3 cases with significantly elevated values were the following

1 A woman of 51 years with systemic lupus erythematosus and sicca syndrome. At the time the blood samples were taken there were obscure abdominal symptoms and high temperature. S-OCT was 0.182 μM S-GOT 104 and S-GPT 95 units. At the follow up 3 years later the patient had no abdominal symptoms, cholegraphy was normal and so were also liver tests, including enzyme levels.

2 A woman of 55 years with systemic lupus erythematosus and fever The findings in all liver tests were strongly pathologic. S-OCT 0.221 μM and S-GOT 52 units.

3 A woman of 64 years with systemic lupus erythematosus and suffering from pronounced fatigue. During the time at the hospital S-OCT rose to 0 174 μM and then returned to normal. Elimination of bromsulphalein was pathologically delayed.

b) *Dermatomyositis* Two of the 3 cases of dermatomyositis (24) had elevated S-OCT All 3 patients were very ill and had greatly increased transaminase levels. The enzyme course for the one with normal S-OCT is shown in fig 8.

c) *Scleroderma.* Three cases of scleroderma showed normal S-OCT

d) *Polymyalgia rheumatica* (24) The 2 patients in this group recorded normal S-OCT

e) *Polyarteritis nodosa* Of the 2 patients in this group one had a normal S-OCT

[1] Of the 238 patients under the headings *Rheumatoid arthritis Systemic lupus erythematosus and allied conditions* and *Other diseases with joint and muscle symptoms,* 206 have been described by Malmqvist and Reichard (24)

and the other a significantly elevated level. At the autopsy of the latter patient pronounced pathologic changes in the liver were found.

f) *Obscure collagenosis* Of the 4 cases of collagen disease which could not be assigned to any of the above groups, 2 had probably elevated S-OCT and 2 significantly elevated levels. Three also had increased transaminases. These cases have been reported in detail elsewhere (24)

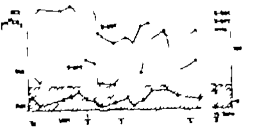


Fig 8. S-OCT S-GOT and S-GPT for a 35-year-old woman with severe dermatomyositis. Note the elevated transaminases which indicate extensive muscle damage and normal S-OCT The shaded area denotes the normal enzyme activities.

Other diseases with joint and muscle symptoms

The patients in this collective group consisted of 50 cases of post-infectious polyarthritis (24) 19 of uropolyarthritis (24) 4 of arthritis psoriatica, 28 of arthrosis, 25 of arthralgiae and 15 of tendinitis and allied conditions. Out of these 141 patients 132 had normal S-OCT levels and 5 probably elevated values. The 4 cases with significantly elevated levels were the following

1 A woman of 55 years with spondyloarthrosis. For one sample S-OCT was 0.132 μM, S-GOT was normal and S-GPT 55 units. The other liver tests gave normal findings. At a follow-up 8 months later the enzyme values and liver tests were normal.

2. A 28-year-old woman with symptoms from the finger joints and the flexor tendons of the hands. S-OCT was 0.125 μM, and S-GOT and S-GPT were within the normal range. All the enzyme values decreased gradually and S-OCT was normalized

3. A man of 40 years with severe arthritis psoriatica. S-OCT was 0 173 μM, S-GOT and S-GPT were normal. It was not possible to follow up this patient.

4. A very obese woman of 53 years with arthrosis in both knee joints. S-OCT was 0.114 μM. At follow-up 4 years later the enzyme values and liver tests were normal.

Gastric and duodenal ulcer and gastritis

The 15 patients in this group comprised 8 with gastric ulcer 3 with duodenal ulcer one with both gastric and duodenal ulcers and 3 with ulcer disease that, however could not be verified radiographically All but one of them had normal S-OCT The exception was a woman of 66 years with a gastric ulcer who showed a value of 0 066 μM (probably elevated) Nine other patients with some other dominating disease concurrently with the ulcer had normal S-OCT

Diseases of the small intestine

Two patients with malabsorption syndrome of uncertain aetiology had normal S-OCT Eight out of 14 cases of acute gastroenteritis had normal S-OCT and 4 had probably elevated levels. The 2 with significantly elevated S-OCT were the following

1 A woman of 78 years who, after diarrhoea with bloody stools lasting 5 days, was admitted to hospital with good general status. Three days after the diarrhoea had ceased the S-OCT rose from normal to 0 115 μM. Liver tests and transaminases were normal throughout.

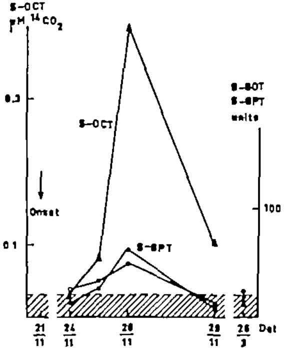


Fig 9 S-OCT S-GOT and S-GPT for a 25-year old male with fever and frequent diarrhoea two days prior to admission. Thymol turbidity serum bilirubin, serum paper electrophoresis and alkaline phosphatase activity were normal. At a follow-up examination 4 months later the liver enzyme activities in serum and other liver tests were normal. The shaded area denotes normal S-OCT level.

2. A 25-year-old man with high temperature and bloody diarrhoea lasting for 2 days. Five days after the onset, when the symptoms had disappeared, S-OCT was elevated at 0 405 μM, S-GOT at 51 and S-GPT at 61 units. Other liver tests were normal. At a follow up 4 months later the liver tests and serum enzyme values were normal (fig 9)

As has been described elsewhere (6) the raised S-OCT values in these cases of acute enteritis were not related to the frequency of diarrhoea, presence of blood in the faeces, the general status or the results of the usual liver tests. Most elevated values were observed 2—4 days after the onset of the diarrhoea.

Ulcerative colitis

Of the 11 cases of ulcerative colitis all but one had normal S-OCT

A 23-year-old man with severe symptoms of many years duration recorded a level of 0 400 μM. At a follow-up 3 years later both enzyme and liver tests were normal.

Blood diseases (not malignant)

Normal S-OCT levels were recorded for 7 cases of haemolytic anaemia, 2 of thrombocytopaenia, 2 of vitamin B_{12}-deficiency anaemia, 2 of polycythaemia and one each of sidéropaenic anaemia, absence of globulin in the blood and haemophilia

Miscellaneous diseases and conditions

In 66 of the 76 patients assigned to this group normal S-OCT levels were recorded The values were probably elevated in 4 and significantly elevated in 6.

The 66 cases with normal S-OCT comprised 12 of psychoneurosis, 10 observation cases, 8 each of chronic nephritis with uraemia and diabetes mellitus, 4 of bronchial asthma 3 each of obliterating peripheral arteriosclerosis and cerebral apoplexy 2 each of acute pneumonia and thrombophlebitis, and one each of pulmonary tuberculosis, pleural empyema essential hypertension toxic goitre, hypogonadism craniopharyngeoma subphrenic abscess, chronic pancreatitis, achalasia of cardia, branchial cyst, multiple sclerosis, diffuse calcinosis of the tendons and ligaments, lymphogranulomatosis benigna and nodular non suppurative panniculitis.

S-OCT was probably elevated in 2 cases of acute pneumonia, one of bronchial asthma and one of obesity

The 6 cases with significantly elevated S-OCT consisted of the following

1 A woman of 68 years suffering from diabetes with complicating nephropathy and obscure attacks of fever S-OCT was 0.330 μM The patient died subsequently no autopsy was performed.

2. A man of 65 years with pulmonary emphysema, herpes zoster and obscure fever S-OCT was 0.140 μM and S-GPT was also elevated. The patient died 2 years later from heart decompensation. The biliary tree and gall bladder were normal.

3. A man of 46 years with recurrent cerebral malacia. S-OCT and S-GPT increased in association with diagnostically obscure pains in the chest. S-OCT was 0.157 μM. At a follow-up 3 years later the serum enzymes and liver tests were normal.

4. A man of 83 years with diagnostically obscure pains in the chest and abdomen, and fever. S-OCT was 1.96 μM, S-GOT was 680 units and S-GPT 180. It was not possible to follow up this patient.

5. A woman of 55 years with aetiologically obscure tachycardia of longstanding (120/min.) S-GOT and S-GPT had been pathologically elevated for long period S-OCT was 0.200 μM. The other liver tests were normal. At follow-up three years later there was no tachycardia and the enzyme and liver tests were normal.

6. A man of 51 years with abdominal pains owing to spontaneous (?) subcapsular renal haemorrhage. S-OCT was 0.233 μM and S-GOT and S-GPT were also pathologically elevated. At follow-up 3 years later the enzyme and liver tests were normal.

Discussion

In the present study as in earlier experiments on the dog (26—28) and man (29, 30) the S-OCT activity was usually elevated where there was evidence of biliary stasis or liver damage. High values were also recorded in most cases of alcoholism, condition known to be followed by acute liver cell damage and elevated enzyme levels (23 31—34) The elevated S-OCT values in many patients with low blood pressure and accelerated pulse rate (shock) or congestive heart failure is in close agreement with the observation of centrilobular cell necrosis of the liver (35—37) and elevated S-GOT and S-GPT activities (38—40) reported in cases of this type However high S-OCT values were noted in some cases of myocardial disease where there were no grave signs of circulatory failure most of these also displayed an increase in S-GPT In a number of these cases morphine had been administered. The increase in biliary pressure due to this drug may result in a raised level of S-OCT (30) and this may account for the high values in some cases of the present series. As regards the other patients, it is conceivable that even moderate circulatory disturbances in the liver may liberate hepatic enzymes into the blood.

S-OCT was elevated in several cases of rheumatoid arthritis, systemic lupus and some other allied collagen conditions. As there was no apparent association between the enzyme level and the form of treatment, the high values were probably due to liver involvement as a component of the disease in some of these cases. In untreated cases of rheumatoid arthritis, Lövgren et al. (41) found that a reduced elimination rate of bromsulphalein was a sign of impaired liver function and a similar observation was made by Nettelbladt (42) in treated cases of rheumatoid arthritis. Some cases of lupus erythematosus with elevated S-OCT in the present series had additional signs of liver damage.

The elevated S-OCT in cases of acute enteritis could be due to leakage of ornithine carbamoyl transferase from damaged intestinal cells, but this is unlikely in view of the fact that the increases in serum enzyme activity appeared 2—6 days after the onset of the disease when its acute phase had passed Furthermore, some of the cases of acute enteritis showed no elevation, nor was the increase associated with the presence of blood in

the stools (6) It seems likely therefore that in these cases too the liver was responsible for the elevations in S-OCT

Among 259 other patients in this series with other than liver disease only 11 had significantly elevated S-OCT 6 of these also had elevated S-GOT and S-GPT The only common source of these elevations seems to be the liver Biliary stasis caused by morphine, circulatory insufficiency or undetected liver disease, such as cirrhosis, might have been responsible for the elevations in S-OCT in these few cases

The results indicate that the determination of S-OCT provides a more sensitive test than S-GOT S-GPT and S-AP for liver damage and biliary stasis of short duration. This confirms our earlier findings (27—30) but is in contradiction to those of Nowak (43) the reason for this discrepancy lies in the greater sensitivity of the technique used in the present study However caution is indicated when comparing the different liver tests of the present study since the normal limits for the methods were not established in the same way

A variety of methods have been evolved for determining the activity of enzymes in serum either when they are distributed throughout the body or when they are restricted to the liver (see, for instance the review by West and Zimmerman (44)) Of the many serum enzyme tests, the determination of S-AP S-GOT and S-GPT are well established easy to perform and inexpensive, and they provide valuable diagnostic information in cases of heart muscle and liver disease In many cases, however these tests are not sufficiently sensitive. This applies to certain cases of temporary stasis of the common bile duct, cirrhosis of the liver carcinoma of the liver circulatory failure with inadequate blood supply to the liver and alcoholism. The transaminases are not specific enough to distinguish between liver damage and muscle disease. Under these conditions the S-OCT determination however provides a useful guide in diagnosis

Summary

In man ornithine carbamoyl transferase is localized mainly in liver and, to a much smaller extent in the small intestine. In normal subjects the activity of this enzyme in serum is extremely low

In order to examine the specificity and sensitivity of S-OCT analyses in the diagnosis of liver and biliary tract disease S-OCT was determined on about 3 000 blood samples from 695 cases of various diseases.

Elevated levels were observed in all cases of infectious hepatitis, acute biliary pains, jaundice due to gall stones, tachycardia with lowered blood pressure more than 75 per cent of the cases of alcoholism, of liver cirrhosis and jaundice due to malignant disease, more than 50 per cent of the cases of primary or secondary carcinoma of the liver (without jaundice) and of congestive heart failure in more than 25 per cent of the cases of malignant systemic diseases, systemic lupus erythematosus and allied collagen conditions, and of acute enteritis in some cases of myocardial disease without signs of heart decompensation and in 9 out of 67 cases of rheumatoid arthritis.

Normal S-OCT levels were recorded in almost all of the remaining 259 cases of diseases and conditions other than those of the liver and biliary tract Extremely high S-OCT levels were observed in early cases of infectious hepatitis some of the cases of decompensated cirrhosis of

the liver some of acute biliary pains, and in biliary stasis during the first days of the condition. Extremely high S-OCT levels were recorded also in irreversible shock.

It would seem that the liver was the source of increased leakage of ornithine carbamoyl transferase into the blood stream, although in cases of acute enteritis the increase in S-OCT may have been due to damage to cells of the small intestine.

The findings of the present study indicate that S-OCT is a more sensitive indicator of liver damage and temporary biliary stasis than S-GOT S-GPT and S-AP

In most cases of liver and biliary tract disease, the common, simple and less expensive liver tests are accurate enough for diagnostic purpose. In some cases of temporary biliary stasis, liver cirrhosis, carcinoma of the liver and in circulatory failure and alcoholism the conventional liver tests may not be sensitive enough. The transaminases are not specific enough to distinguish between liver damage and muscle disease. Under these conditions the S-OCT determination, however provides a useful guide in diagnosis.

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Among 259 other patients in this series with other than liver disease only 11 had significantly elevated S-OCT 6 of these also had elevated S-GOT and S-GPT The only common source of these elevations seems to be the liver Biliary stasis caused by morphine circulatory insufficiency or undetected liver disease, such as cirrhosis might have been responsible for the elevations in S-OCT in these few cases

The results indicate that the determination of S-OCT provides a more sensitive test than S-GOT S-GPT and S-AP for liver damage and biliary stasis of short duration. This confirms our earlier findings (27—30) but is in contradiction to those of Nowak (43) the reason for this discrepancy lies in the greater sensitivity of the technique used in the present study However caution is indicated when comparing the different liver tests of the present study since the normal limits for the methods were not established in the same way

A variety of methods have been evolved for determining the activity of enzymes in serum either when they are distributed throughout the body or when they are restricted to the liver (see, for instance, the review by West and Zimmerman (44)) Of the many serum enzyme tests the determination of S-AP S-GOT and S-GPT are well established easy to perform and inexpensive, and they provide valuable diagnostic information in cases of heart, muscle and liver disease In many cases, however these tests are not sufficiently sensitive This applies to certain cases of temporary stasis of the common bile duct cirrhosis of the liver carcinoma of the liver circulatory failure with inadequate blood supply to the liver and alcoholism. The transaminases are not specific enough to distinguish between liver damage and muscle disease. Under these conditions the S-OCT determination however provides a useful guide in diagnosis.

Summary

In man ornithine carbamoyl transferase is localized mainly in liver and to a much smaller extent, in the small intestine. In normal subjects the activity of this enzyme in serum is extremely low

In order to examine the specificity and sensitivity of S-OCT analyses in the diagnosis of liver and biliary tract disease, S-OCT was determined on about 3 000 blood samples from 695 cases of various diseases.

Elevated levels were observed in all cases of infectious hepatitis acute biliary pains, jaundice due to gall stones, tachycardia with lowered blood pressure more than 75 per cent of the cases of alcoholism, of liver cirrhosis and jaundice due to malignant disease, more than 50 per cent of the cases of primary or secondary carcinoma of the liver (without jaundice) and of congestive heart failure, in more than 25 per cent of the cases of malignant systemic diseases, systemic lupus erythematosus and allied collagen conditions, and of acute enteritis, in some cases of myocardial disease without signs of heart decompensation and in 9 out of 67 cases of rheumatoid arthritis.

Normal S-OCT levels were recorded in almost all of the remaining 259 cases of diseases and conditions other than those of the liver and biliary tract. Extremely high S-OCT levels were observed in early cases of infectious hepatitis, some of the cases of decompensated cirrhosis of

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Myotonic Human Musculature: Stimulation with Depolarizing Agents

I. Mechanical Registration of the Effects of Acetylcholine and Choline

By

Gustav Örndahl

The myotonic phenomenon is defined as a disorder of the striated voluntary muscle tissue in which the relaxation does not pass off, as it normally does, in a fraction of a second, but continues for several seconds (27). This phenomenon may occur as the only symptom in cases of myotonia congenita (m. c.) or it may arise in connection with other symptoms in cases of dystrophia myotonica (d. m.). It can be brought about voluntarily or by mechanical or electrical stimulation of the muscle. There are three types of reactions: the active (or voluntary), the mechanical (or percussive), and the electrical myotonic reaction (27, 38, 48).

A review of the literature about neuromuscular transmission has been published by del Castillo (11). Valuable information has been obtained by studying nerve-muscle preparations from different animals, with or without administration of acetylcholine (ACh) injected intra-arterially or given by means of micro-pipettes directly into the motor end plate. With the use of micro-electrodes it is possible to register potentials in the motor end plate or in the muscle membrane. It is generally acknowledged that ACh is the transmitter substance between the motor nerve and the motor end plate. In the end plate ACh causes depolarization which is followed by repolarization until the resting potential has been restored. The end plate contains cholinesterase which rapidly breaks down ACh to choline (Ch) and acetate. This prevents a blocking effect by ACh on the nerve muscle transmission. With depolarization there arises a potential (end plate potential) which, on reaching a critical amplitude, propagates along the muscle fibre (action potential) causing a brief contraction in the fibre. The electrical activity associated with

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whether the reaction of myotonic muscle to intra-arterial injection of these agents differs from that of healthy muscle. No investigation of the effect of Ch on myotonic musculature has been published previously

Review of the literature

The effect of intra-arterial injections of ACh was studied by Lanari (31) in six patients with myotonia, and by Engbaek (15) in one patient with m. c. (table I) Of Lanari's six patients, three had atrophy and two hypertrophy while one had neither atrophy nor hypertrophy of the musculature. These authors did not use an objective registration method, nor did they analyse the results extensively. Experiments with intra-arterial injections of ACh have been carried out on healthy subjects and on patients who had myasthenia gravis (1 15 22, 23 24 25 51) Table I gives published data on the stimulating effect of ACh in healthy subjects and in patients with myotonia. It is evident from the table that ACh causes fasciculations and finger flexion in healthy subjects and that its effects vary greatly in different subjects. Grob et al. (22) in testing ACh in doses from 2 to 10 mg noted an increase of the duration and intensity of the effect. When the dose was further increased to 40 mg, they observed an effect in some subjects but none at all in others. Acheson et al. and Grob et al. found that even when equal doses were injected into the same subject at intervals of from 1/2 minute up to several days, the effects varied greatly. Acheson et al. (1) registered the effect mechanically. Grob et al. electromyographically while the other authors listed in the table used no registration procedure, but simply observed involuntary movements of the muscle.

Grob et al. (22) studied the muscular response to intra-arterial injections of Ch in twelve healthy subjects. This is the only report which could be found in the literature concerning the effect of Ch on human musculature.

Material

The investigation was made on two groups of volunteers a group with d. m. and a control group without muscular or neuromuscular diseases.

The group with d. m. consisted of two men and one woman (54 43 and 25 years of age) In addition to muscle symptoms, the patients had one or more of the other classical symptoms, e.g. mental deterioration, reduced B. M. R., and d. m. heredity. One of the men had testicular atrophy and baldness. The musculature of the forearm and of the hand showed an active and mechanical myotonic reaction and characteristic electromyographic features (39). Other symptoms were paresis and atrophy of the sternocleidomastoid muscles and of the extremital muscles. Repeated determinations of serum Na, Ca, Mg, Cl and CO_2 showed normal values. Seven K values were within the normal range (3.6—5.1 mEq/l) and four K values above the limit for normal (5.2, 5.4 5.6 and 6.0 mEq/l) All patients were examined with depolarizing muscle relaxants on another occasion (53)

The control group consisted of two men and one woman (34 51 and 58 years of age) without previous or current clinical muscular or neuromuscular symptoms. At the time of the investigation they were under treatment for arterial hypertension or obesity

Methodology

The ACh and Ch studies were usually done without administration of any other agent. Only one subject — a d.m. patient tested with large doses of ACh — required general anesthesia (53) to preclude the pain associated with a pronounced muscular response.

Table I Published data on stimulating muscular effect of acetylcholine given in the brachial artery of healthy subjects and of patients with myotonia

Author and year	No. of cases	Dose variations (mg)	Effect
I *Healthy subjects*			
Lanari (1936)	Not stated	40	None
Harvey et al. (1941)	20	10—50	In 5 subjects fasciculations, brief, insignificant finger flexion. Duration about 20 sec.
Wilson & Stoner (1947)	13	40	In 8 subjects fasciculations or finger movements. Duration (stated only for 1 subject) 10 sec.
Acheson et al (1948)	5	0.04—12.5 0 1—1.0	In 4 subjects mechanically registered contraction. Duration given only in a fig maximum about 45 sec.
Engbaek (1951)	33	0 1—0.35	In all subjects, flexion of one or more fingers. Duration about 1 sec.
Grob et al. (1956)	35	0.1—2	In all subjects fasciculation and slight flexion twitch of fingers. Duration 5—15 sec.
		2—10	More sustained flexion of fingers and hand.
		25—40	Little or no movement
II *Patients with myotonia*			
Lanari (1936)	6	40	In all patients, contracture. Duration about / min.
Engbaek (1951)	1	0.005—0.250	Tonic contraction. Duration 1—2 min.

Smallest effective dose.

this contraction may be registered by means of electromyography The term contracture, in the physiological and pharmacological sense of the word has been defined by Gasser (19) It denotes a more prolonged shortening or increased tension of the muscle. This response is not associated with action potentials and does not give rise to electrical activity (spikes) during electromyographic registration. Contracture, too results from depolarization (29) and like contraction, it is reversable; it can involve the entire muscle fibre or only a part of it.

Most authors (10 32 33 37 40) maintain that myotonia is caused by changes in the muscle fibre while others (18, 21 46) hold that the disturbance is confined to the motor end plate. The mechanism has not been conclusively established.

The purpose of this investigation is to shed additional light on the active myotonic phenomenon by administration of depolarizing agents to myotonic musculature. Since ACh is the transmitter substance at the motor end plate and Ch is a precursor and decomposition product of ACh it should be of interest to ascertain

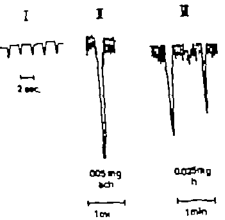


Fig. 1. Dystrophia myotonica. Examples of isometric registration of: I. The indirect muscular twitches upon supramaximal electrical nerve stimulation; II. The response after acetylcholine evidenced by deep deflection (0.05 mg, the smallest effective dose) and III. The response after choline (two injections of 0.025 mg, the smallest effective dose). The apparatus produces downward registration.

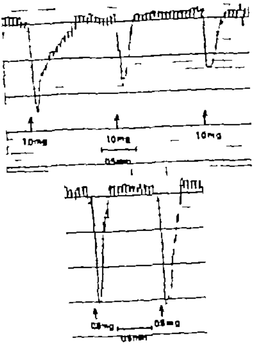


Fig. 2. Dystrophia myotonica. Isometric registration. Varying responses of patient to different doses of acetylcholine (1 mg and 0.5 mg)

Results

When the ulnar nerve was electrically stimulated, a flexion of the fingers or a change of tonus in the flexor muscles (twitch) occurred. Patients with d. m. exhibited relatively protracted flexion or a change of tonus (mechanical response) after ACh and Ch, while neuromuscularly healthy controls showed a response only after injection of ACh. Table II shows the number of injections which elicited a registrable muscular response.

I. PATIENTS WITH DYSTROPHIA MYOTONICA

1 Acetylcholine

After injection of ACh the three patients usually showed an involuntary mechanical response of the flexor muscles of one or more fingers. The response was registered isotonically or isometrically. The tracing corresponded most closely to the twitch which occurs on stimulation of the nerve (fig. 1). In those cases in which a pronounced muscular response was observed, the tracing differed somewhat (first injection, fig. 2).

The intensity and duration of the mechanical response after ACh injection varied greatly in different patients and in individual subjects both during single investigations and from study to study. Attempts to determine the smallest effective dose also yielded varying results. In one experiment a subject showed no muscular response to four doses of 0.2 mg given in series — or to lesser doses — but responded to other doses of 0.2 mg and more. On another occasion the same patient responded to some doses of 0.25 mg and 0.5 mg, but showed no

Table II Patients with dystrophia myotonica and neuromuscularly healthy controls. Pharmacological tests

Agent	No. of subjects	No. of series	Dose variations (mg)	Total no. of injections	No. of injections with a positive result	Relative frequencies with a positive result
Dystrophia myotonica, investigated without anesthesia						
Acetylcholine	2	4	0.025—3	169	126	74.6
Choline	2	2	0.025—10	66	47	71.2
Dystrophia myotonica investigated with anesthesia						
Acetylcholine	1	1	20—25	7	7	100.0
Controls, investigated without anesthesia						
Acetylcholine	3	3	0.05—5	33	3	9.1
Choline	3	3	0.05—40	52	0	0.0

Results were regarded to be positive when a mechanical response was registered. Unlike myotonia patients the controls showed only slight responses.

Administration of the agents

Inasmuch as ACh is unstable when dissolved, the solution was prepared immediately prior to the examination (Acetylcholine chloride, Hoffman-la Roche). Ch, on the other hand is stable and the solution was usually prepared a day or two before the examination (Choline chloride, Merck). Control tests were made with physiological saline. The injections were given as rapidly as possible through a polyethylene catheter inserted in the proximal direction in the brachial artery at elbow level. The circulation was at no time interrupted. The volume of the intra arterial injection was, as a rule 1 or 2 ml. The concentration of the injected solution varied. The catheter contained about 0.2 ml. It was not rinsed after the injection. In order to preclude error associated with the catheter volume, at least two injections were always given at equal concentrations. The effect of the first injection is omitted from the discussion of the results. The exact doses entering the circulation are included in the presentation of the results. Table II shows the total number of injections of ACh and Ch given to patients with d. m. and to the controls as well as the doses used.

Nerve stimulation

The ulnar nerve was stimulated in its sulcus at the elbow. A small electrode was fixed over the nerve and a larger one was positioned on the upper arm. The electrodes were connected to a Grass stimulator which had been adjusted so as to give rectangular current impulses with a duration of about 3 milliseconds. The frequency of stimulation was, as a rule, 16—18 per min. The voltage was adjusted to elicit a maximal muscle response. Stimulation of the ulnar nerve was continued throughout the experiment.

Registration

The forearm and hand were fixed to the support in supination, the fingers partially flexed. The movements of the fourth and/or fifth finger or the changes of tonus in the musculature of these fingers, were registered by means of a kymograph. Isotonic registration was done in accordance with the method described by Thesleff (47). For isometric recording an apparatus described by Malmström (34) was employed. The latter method was applied in four series of tests on two patients with d. m. and in one series on a control. In all other investigations the first method was employed.

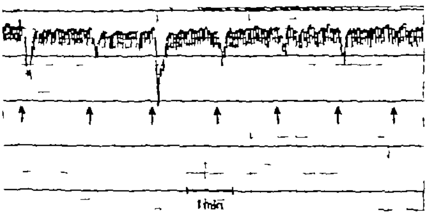


Fig. 5. Dystrophia myotonica. Isometric registration of the varying responses to repeated equal doses of choline (10 mg).

10 mg did a response occur. Another patient twice responded to injections of 0.025 mg Ch (fig. 1) while six other injections of the same dose produced no reaction. The smallest effective dose of ACh in this patient on this occasion was 0.05 mg (fig. 1). Increasing doses up to 10 mg — with the exception of some injections of 0.125 mg — invariably elicited a response.

As is evident from the isometric curves (fig. 5) the effect sometimes increased and sometimes decreased in the same patient with repeated doses of 10 mg at intervals of 1—2 min. As with ACh, the duration of the mechanical response to injections of Ch varied considerably. In some experiments a duration of up to 30 sec. was measured (0.1 mg).

As may be seen in figs. 2 and 3, the twitch did not vary to the same degree as did the muscular response to ACh and Ch, the latter effect being invariably more substantial than the twitch. This could be explained by the participation, in the response, of muscle groups other than those innervated by the ulnar nerve. The stimulation, moreover, had a longer duration.

II. CONTROLS

1. Acetylcholine

After 30 ACh injections there was no mechanical response. Only after three injections (1 mg, 1 mg, 5 mg) was there a brief involuntary mechanical response of the flexor muscles of one or more fingers in two of the three controls. These doses were frequently painful.

2. Choline

Injection of Ch elicited no mechanical response in any instance although one of the three controls received 35 injections in doses up to 40 mg.

Both in patients with d. m. and in the controls redness of the skin and sweating peripheral to the site of injection were observed after injection of ACh. No such reactions were noted after injection of Ch.

Injections of physiological saline solution elicited no mechanical response in patients with d. m. or in the controls.

Discussion

The results of these investigations showed great variations in the responses of d. m. patients to ACh and Ch. According to the literature reviewed the

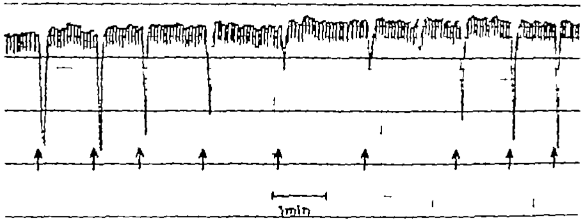


Fig 3. Dystrophia myotonica Isometric registration of the varying responses to repeated equal doses of acetylcholine (0 5 mg)

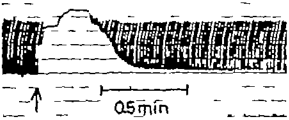


Fig 4 Dystrophia myotonica Isotonic registration of the effect of acetylcholine (25 mg) The apparatus gives an upward registration.

reaction to other injections of 0.25 mg and 0 5 mg Doses exceeding 0 5 mg however invariably elicited a response. In one experiment eight injections of ACh in increasing doses up to 1 mg were given to another patient without demonstrable effect. No mechanical response was registered until the ninth injection of 1 mg Later on in the experiment, doses of 0.20 mg 0 25 mg and 0.50 mg were given and a definite response was elicited each time. In a further test series the same patient exhibited a pronounced mechanical response to 0 05 mg and to other doses of up to 1 mg but did not react to 0 025 mg The effects of varying doses may be seen in fig 2.

The varying effects of equal doses are given in fig 3 which shows that the response sometimes increased and sometimes decreased during the course of the tests. The duration of the mechanical response following equal injections was measured and in some cases, was found to vary considerably After injections of 1 mg ACh the duration of the mechanical response varied between 15 sec. and 45 sec. (fig 2) The same dose at a different concentration gave a duration of about 2 min.

The patient who was studied under general anesthesia received seven ACh injections and reacted to each with a mechanical response, usually of 35—45 sec. duration (fig 4) The interval between the injections was 2—5 min. The response increased initially then decreased.

° *Choline*

Injection of Ch was usually followed, in both patients, by an involuntary mechanical response of the flexor muscles of one or several fingers. The response was registered isometrically With this agent too there were great variations in the registered response. In one experiment doses between 2 mg and 8 mg were without effect and only after doses of

Table III. Published data on stimulating muscular effect of acetylcholine (ACh) and choline (Ch) in animals

Author and year	Muscle/animal	Agent	Administration	Effect
I. *Amphibians*				
Gasser & Dale (1926)	Rectus abdominis (frog)	ACh	Muscle bath	Contracture
Brown (1937) (7)	Innervated and denervated gastrocnemius (frog)	ACh	Close arterial injection	
		Small dose		Contraction
		Large dose		Contraction and contracture
Fleischer et al. (1960)	Rectus abdominis (frog)	Ch	Muscle bath	Contracture
II. *Birds*				
Brown & Harvey (1938)	Innervated and denervated gastrocnemius (hen)	ACh	Close arterial injection	
		Small dose		Contraction
		Large dose		Contraction and contracture
III. *Mammals*				
a) Foetuses and new-born animals				
Rückert (1930)	Rectus abdominis, pectoralis and others (guinea-pig, rabbit, dog)	ACh	Local application	Contracture
b) Adult animals				
Dale & Gasser (1926)	Denervated gastrocnemius (cat)	ACh, derivatives of or analogous to Ch	Intra-arterial injection	Contracture
Brown et al. (1936)	Innervated gastrocnemius (cat)	ACh	Close arterial injection	Twitchlike contraction
Brown (1937) (6)	Innervated gastrocnemius (cat)	ACh	Close arterial injection	Asynchronous tetanus
Brown (1937) (7)	Denervated gastrocnemius (cat)	ACh	Close arterial injection	
		Small dose		Contraction
		Large dose		Contraction and contracture
Bacq & Brown (1937)	Innervated gastrocnemius (cat)	Ch	Close arterial injection	Contraction

same observation has been previously recorded in connection with intra arterial injections of ACh in healthy subjects. Most of the authors (15 22 25) administered ACh into the brachial artery after interrupting the circulation. In the present investigation, however the agent became mixed with that quantity of blood which passed the injection site during administration. The variations in the muscle response could be ascribed to changes occurring in the supply of ACh when the intra-arterial injection site is not close to the investigated musculature. Factors of significance in this connection have been discussed by Acheson et al. (1) in conjunction with experiments on humans and by Rowley et al. (43) in experiments on animals The latter authors administered nitroglycerin agents to maintain maximum dilatation of the muscle vessels.

After some ACh injections in patients with d m in this investigation, there occurred a mechanical response of considerably longer duration (1—2 min.) than that cited in the literature for normal subjects (1—15 sec.) A similar finding was made by Engbaek (15) in a patient with m. c. who received an injection of 0 005 mg ACh. Lanari (31) obtained a duration of 1/2 min. after injection of 40 mg ACh in patients with myotonia The lowest dose which elicited a mechanical response in my patients with d m. was 0 05 mg ACh Engbaek recorded a response in a patient with m. c. after as small a dose as 0 005 mg The lowest effective dose mentioned in the literature for healthy subjects is 0 1 mg An increased ACh sensitivity in patients with amyotrophic lateral sclerosis and peripheral neurogenic paresis, i e diseases involving injury to the peripheral motor neuron, was noted by Engbaek (15)

Due to the great variations in ACh sensitivity in healthy subjects as well as in patients with d m., it is difficult to determine whether myotonic muscle is more sensitive to the influence of ACh than normal muscle. In view of this consideration it was decided to test the muscular reaction to Ch which has considerably less effect than ACh. The stimulating effect of Ch on mammalian muscle is about 1 000 times less than that of ACh (3) Del Castillo and Katz (12) observed by iontophoretic application of Ch and ACh to the receptors in the end plate that the depolarizing capacity of Ch was less than one hundredth that of ACh.

In my experiments with Ch, a mechanical response of the muscle was recorded in both patients with d m., one of whom reacted to a dose as small as 0 025 mg In the three controls, on the other hand no mechanical response was recorded after doses of up to 40 mg Grob observed (personal communication) that Ch given intra arterially causes a sustained muscular contraction in patients with d. m. Grob Johns and Harvey (22) studied the stimulating effect of Ch in doses between 5 and 100 mg in 12 healthy subjects. A brief period of visible fasciculations was noted (personal communication from Johns) In my experiments patients with d m showed a muscular response to both ACh and Ch administered in small equal doses (0 05—1 mg) This observation is noteworthy since the stimulating effect of Ch on mammalian muscle, as mentioned above, is considerably less than that of ACh. My experiments indicate that the muscle in d m is more sensitive than normal muscle to the stimulating effect of Ch Patients with myotonia show an increased sensitivity to Ch, the active decomposition

tivity of the muscle fibre to ACh (50). Repeated electrolyte determinations in the serum of the myotonia patients investigated here, showed values within the normal range, though some serum potassium values above the upper limit of normal were observed. Similar findings have been reported by other authors (36, 37, 45, 49). Since increased serum potassium values have been observed in other diseases unrelated to myotonia, and since they are not typical for myotonia, the active myotonic phenomenon could scarcely be attributed to an electrolytic disturbance in serum.

Since myotonic muscle reacts to Ch in the same manner as does denervated mammalian muscle, it is reasonable to suspect the presence of anatomical or functional injury to the peripheral motor nerve in cases of myotonia. Wohlfart (52) was unable to demonstrate any microscopic change in the spinal nerves of d. m. cases. A histological study of biopsies from the accessorius nerve branch supplying the sternocleidomastoid muscle, which in d. m. is extremely atrophic, also failed to reveal any change (unpublished observations, Brattgård and Örndahl). Investigations designed to measure the velocity of conduction in peripheral nerves in d. m. cases are now in progress. Five patients have been studied thus far. The values obtained have varied between 43 and 60 m/sec. (mean 51) for the ulnar nerve and between 32 and 44 m/sec. (mean 38) for the peroneal nerve (unpublished observations, Steg and Örndahl). Normal values for the method used are 49—65 m/sec. (mean 57) for the ulnar nerve and 38—50 m/sec. (mean 44) for the peroneal nerve. In patients with diabetes mellitus, with or without neuropathy, Fagerberg et al. (16) found peripheral nerve conduction velocities equal to or lower than those observed in cases of d. m. None of these patients, however, showed symptoms of myotonia (personal communication from Fagerberg). It seems unlikely, therefore, that the active myotonic phenomenon is caused by anatomical or functional injury to the peripheral nerves.

A few authors have compared myotonic and denervated muscle. Landau (33) in a report on the mechanism in cases of myotonia, writes: "The essential nature of peripheral myotonia is highly similar to that of denervated muscle." Ravin (41) states: "The denervated muscle and myotonic muscle show remarkable similarities." He further observes: "The most remarkable similarity is the occurrence of prolonged contraction after a period of rest. This is seen in the myotonic muscle after contraction produced by nerve impulses or by mechanical or electrical stimuli. In the denervated muscle this prolonged first contraction occurs after electrical stimulation." He continues: "The prolonged contraction seen in denervated muscle is influenced by several drugs in almost exactly the same manner as the myotonic contraction." The agents used by Ravin (41) were epinephrine, quinine, calcium, potassium and eserine. He pointed out that, in these conditions, there is an increased irritability to mechanical stimulation, galvanic current and to ACh. His histological comparisons disclosed similarities between the two conditions, i. e., corresponding changes in the sarcolemmal nuclei, and the presence of nuclei within the muscle fibres. These observations as respects d. m., have been confirmed by other authors (2). Atrophy of the muscle is not found in cases of m. c. but does occur in d. m. patients. It must here be em-

Table III (cont.)

Author and year	Muscle/animal[1]	Agent	Administration	Effect
Rosenblueth & Luco (1937)	Denervated gastrocnemius (cat)	ACh	Close arterial injection	
		Small dose		Contraction
		Large dose		Contraction and contracture
Masland & Wigton (1940)	Innervated muscle (cat)	ACh	Close arterial injection	Fasciculations and contractions
Hutter (1952)	Innervated tibialis anterior (cat)	Ch	Intravenously Close arterial injection	Fibrillations Fibrillations and contractions
Blaber & Bowman (1959)	Innervated tibialis anterior (cat)	Ch	Close arterial injection	Fasciculations and potentiations of the indirect twitch
	Denervated tibialis anterior (cat)	Ch	Close arterial injection	Contracture

There is an increased sensitivity to ACh in denervated frog and bird muscle (7 9) and in denervated mammalian muscle (7 13, 28)

product of ACh and the same seems to be the case when ACh is administered (table II)

Pipberger (38) who compiled studies of myotonic muscle, maintains that no difference between myotonic and healthy human muscle has been observed in pharmacological experiments. My investigations indicate, however that myotonic muscle does not react to Ch in the same manner as does healthy human muscle.

Certain animal muscles react in a manner unlike that of healthy human muscle. Table III shows some of the results from reported animal experiments in which the stimulating effects of ACh and Ch were studied The table shows that certain frog and bird muscles in healthy and in denervated state as well as foetal and denervated mammalian muscle react to ACh with a contracture, and that frog muscle and denervated mammalian muscle show a similar response to Ch.

The myotonic muscles studied in this investigation reacted to Ch with a mechanical response similar to that observed in frog and denervated mammalian muscle.

Table III further shows that healthy musculature of adult mammals sometimes contracts in response to ACh and Ch The myotonic musculature investigated here showed the same mechanical reactions to ACh and Ch. Whether this response constituted a contraction or a contracture or a combination of the two, is a question which has been subjected to investigation and which will be dealt with in a subsequent paper (39)

It is known that the membrane potential in muscle fibre is affected by electrolytes A change in the extracellular potassium concentration modifies the sensi

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3 In patients with dystrophia myotonica, acetylcholine resulted in a muscular response of considerably longer duration (1—2 min.) than that mentioned in the literature for healthy subjects injected with the same dose (1—15 sec.)
4 The myotonic muscle reaction to choline injections did not correspond to that of healthy human muscle, but approximated that of healthy frog muscle and denervated mammalian muscle. This observation has not been previously demonstrated.
5 The investigation has shown that myotonic muscle is more sensitive to the stimulating properties of acetyl choline and choline than is healthy human muscle.

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The myotonic musculature in three patients with dystrophia myotonica was studied *by means of stimulation with* acetylcholine (176 injections) and choline (66 injections) Control experiments (33 and 52 injections, respectively) were made on three neuromuscularly healthy subjects. The injections were given in the *brachial artery while circulation was in* progress. The patients and the controls were conscious when studied and no additional agents were used except in one case where anesthesia was induced Recording was isotonic or isometric.

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Myotonic Human Musculature: Stimulation with Depolarizing Agents

II. A Clinico-Pharmacological Study

By

Gustav Orndahl

The myotonic phenomenon is defined as a disorder of the striated voluntary muscle tissue in which the relaxation does not pass off, as it normally does, in a fraction of a second but continues for several seconds (19). This phenomenon may appear in humans as the only symptom in cases of myotonia congenita (m.c.) or it may occur in conjunction with other symptoms in cases of dystrophia myotonica (d.m.). It may be elicited either by voluntary movements or by mechanical or electrical stimulation of the muscle. There are three types of reactions the active (or voluntary) the mechanical (or percussive) and the electrical myotonic reaction (19, 30, 40).

Whether the myotonic phenomenon is caused by changes in the motor end plat or in the muscle fibre itself has not been determined.

It is generally acknowledged that acetylcholine (ACh) is the transmitter substance between the motor nerve and its muscle. Del Castillo and Katz (7) considered that there is no basis for differentiating between the effects of exogenous and of endogenous ACh on the end plate. The decomposition product of ACh, choline (Ch) has — like ACh — a stimulating effect on mammalian muscle, but a far less pronounced one (about 1,000 times less (2)). The following depolarizing muscle relaxants succinyldicholine (SCh) and decamethonium (C_{10}) affect the end plate in essentially the same way as does ACh (36, 37). They exert first a stimulating effect and then a blocking effect. The same (6) is true of the decomposition product of SCh, succinylmonocholine (SmCh).

The active myotonic phenomenon caused by endogenous ACh may thus be stud-

Depolarization means reduction of the rest ing potential in the end plate or the muscle membrane.

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Table III. Pharmacological tests. Patients with dystrophia myotonica or myotonia congenita (fig. in brackets)

Agent	No. of patients	No. of series	No. of injections	Dose variations (mg)	Mechanical response
Depolarizing agents					
Succinyldicholine	15 (3)	32 (3)	191 (10)	5—250	All patients in each series
Succinylmonocholine	2	3	17	10—390	All patients in each series
Decamethonium	5	5	20	1—5	All patients in each series
Acetylcholine.	8 (2)	12 (2)	216 (14)	0.025—25	All patients in each series
Choline	6	6	73	0.025—40	All patients in each series
Total		63	541		
Non-depolarizing agents					
Curare	3	5	8	5—15	None
Gallamine	1	2	4	20—60	None
Total		7	12		

dahl & Stenberg (45) and Petersén, Stenberg & Orndahl (29).

Lanari (24) and Engback (11) gave intra-arterial injections of ACh to seven myotonia patients and observed muscular effect lasting from half a minute to two minutes. No objective method of registration was used, however and the results were not further analyzed. The literature contains a few references by an anesthesiologist to the use of SCh and C_{10} for relaxation of the musculature during surgery (Kaufman (21)). He reported only that SCh and C_{10} produced myotonia. No investigations of myotonic musculature by means of Ch or SmCh injections, however have been previously published as far as I have been able to ascertain.

Material

The series comprised three groups of volunteers, i.e. one myotonia group in which 18 had d.m. and three had m.c., and two control groups, one of which consisted of 28 patients without, and the other of seven patients with, muscular and neuromuscular diseases (tables I and II).

Methodology

Most of the investigations were performed while the patients were under evipan-N_2O anesthesia following premedication with morphine scopolamine or hydromorphone atropine. One investigation was made during block anesthesia and six studies were made without anesthesia.

ACh and Ch were administered intra-arterially. SCh, SmCh and C_{10}, on the other hand were given intravenously. SCh and C_{10} were also administered intra-arterially to six patients with myotonia. During most of the investigations the ulnar nerve was stimulated at the elbow by means of a Grass stimulator in order to elicit — if possible — maximal muscle responses (fig. 1).

Registration from the ulnar fingers was done mechanically — either isotonically (42 subjects; methodology Thesleff, 1952) or isometrically (19 subjects; methodology Malmström, 1957 or Disa electromyograph)

Table I. Material

Diagnosis	No. of persons	Sex		Age, years	
		♂	♀	Range	Mean
Patients with myotonia					
Dystrophia myotonica	18	14	4	24—61	43
Myotonia congenita	3	2	1	32—77	53
Control group without muscular and neuromuscular diseases	28	17	11	16—78	47
Control group with other muscular or neuromuscular diseases					
Primary muscular disease	2	1	1	17—42	30
Neurogenic muscular diseases	5	5	—	32—63	45
Total	56	39	17		

Table II. Some clinical data on patients with muscular or neuromuscular diseases

Diagnosis	No. of persons	Duration of disease (range)	Muscles studied	
			Weakness	Wasting
Patients with myotonia				
Dystrophia myotonica	18	6—25 years	Advanced	Advanced
Myotonia congenita	3	32—77 years	None	None
Control group with other muscular or neuromuscular diseases				
Dystrophia musculorum progressiva (limb girdle)	2	2—13 years	None	None
Amyotrophic lateral sclerosis	1	3 years	Advanced	Advanced
Arteria spinalis anterior syndrome	1	13 years	Advanced	Advanced
Peroneal muscular atrophy	1	>28 years	Advanced	Advanced
Lesion of right plexus brachialis	1	13 weeks 20 weeks	Moderate Slight	None None
Lesion of left plexus brachialis	1	12 days	Paralysis	None

[1] Mean, 14 years.

ied by means of clinico-pharmacological testing of the effects of exogenous ACh and above mentioned closely related agents on the abnormal muscle. By use of varying doses deviations from the normal may be more easily observed and studied.

The basic question to be answered here is whether myotonic muscle differs from normal muscle in its response to these agents and if so whether the active myotonic phenomenon could be explained on this basis.

Preliminary reports on these investigations were published earlier by Stenberg & Örndahl (34) and by Örndahl et al. (44).

This paper represents as far as material, methodology and results are concerned a compilation of previously published reports by Örndahl (43). Örn

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Registration from the ulnar fingers was done mechanically — either isotonically (42 subjects; methodology Theslelf, 1952) or isometrically (19 subjects methodology Malmström, 1957 or Disa electromyograph).

Table I Material

Diagnosis	No. of persons	Sex ♂	Sex ♀	Age, years Range	Age, years Mean
Patients with myotonia					
Dystrophia myotonica	18	14	4	24—61	43
Myotonia congenita	3	2	1	32—77	53
Control group without muscular and neuromuscular diseases	28	17	11	16—78	47
Control group with other muscular or neuromuscular diseases					
Primary muscular disease	2	1	1	17—42	30
Neurogenic muscular diseases	5	5	—	32—63	45
Total	56	39	17		

Table II Some clinical data on patients with muscular or neuromuscular diseases

Diagnosis	No. of persons	Duration of disease (range)	Muscles studied Weakness	Muscles studied Wasting
Patients with myotonia				
Dystrophia myotonica	18	6—25 years	Advanced	Advanced
Myotonia congenita	3	32—77 years	None	None
Control group with other muscular or neuromuscular diseases				
Dystrophia musculorum progressiva (limb girdle)	2	2—13 years	None	None
Amyotrophic lateral sclerosis	1	3 years	Advanced	Advanced
Arteria spinalis anterior syndrome	1	15 years	Advanced	Advanced
Peroneal muscular atrophy	1	>28 years	Advanced	Advanced
Lesion of right plexus brachialis	1	{13 weeks {20 weeks	Moderate Slight	None None
Lesion of left plexus brachialis	1	12 days	Paralysis	None

Mean, 14 years.

ied by means of clinico-pharmacological testing of the effects of exogenous ACh and above mentioned closely related agents on the abnormal muscle By use of varying doses deviations from the normal may be more easily observed and studied

The basic question to be answered here is whether myotonic muscle differs from normal muscle in its response to these agents and if so whether the active myotonic phenomenon could be explained on this basis.

Preliminary reports on these investigations were published earlier by Stenberg & Örndahl (34) and by Örndahl et al (44)

This paper represents, as far as material methodology and results are concerned a compilation of previously published reports by Örndahl (43) Örn-

muscle were noted. This was true of isotonic as well as of isometric registration (tests were made with ACh, SCh and C_{10}) and of intravenous as well as intra arterial administration of SCh and C_{10}. Nor were any differences observed, between the effects of ACh and SCh in d.m. and in m.c. patients.



Fig. 1. Dystrophia myotonica. Mechanical (isotonic) registration of maximal muscle responses (twitches) caused by electrical nerve stimulation. The considerable rise of the base line shows the mechanical response after injection of 10 mg succinylcholine. The reduction of the twitches demonstrates neuromuscular block.

I. MECHANICAL REGISTRATION ALONE

Succinylcholine

In all myotonia patients mechanical responses of the ulnar flexor muscles were registered in each of the test series after injections of SCh (fig 1). Four patients tested with SCh on various occasions at intervals of up to 7 years showed consistently uniform results. Doses of more than 30 mg usually gave rise to flexion of the wrist and "Geburtshelferstellung" (accoucheur's position) of the hand. A mechanical response was also observed to occur in the musculature of the diaphragm and in the extensors and flexors of the toes.

In the healthy controls there was only a flaccid paresis, sometimes preceded by fasciculations. The most extensive test series involved intravenous injections of SCh and isotonic registration in ten patients with d.m. and 21 healthy controls. These two groups always differed significantly in their reactions.

One patient with a plexus injury was examined when the ulnar flexor muscles were moderately paretic and subsequently during almost complete regression. On both occasions intravenous injections of SCh were followed by a definite mechanical response on the paretic side similar to that observed in patients with myotonia. No such mechanical response occurred on the healthy side — where there was only flaccid paresis similar to that found in healthy controls. In one patient with amyotrophic lateral sclerosis, a very slight mechanical response was recorded after half the total number of SCh injections. In none of the other control cases with muscular or neuromuscular diseases, however was a mechanical response observed.

Succinylmonocholine and decamethonium

In all d.m. patients mechanical responses of the ulnar flexor muscles occurred in each of the test series after injections of SmCh and C_{10}. In the healthy controls, however no mechanical response was registered. When equal injections of these agents and of SCh were repeated at intervals of 5—10 minutes, the mechanical esponse became progressively smaller until it finally disappeared completely in a few test series (tachyphylaxis)

Acetylcholine

Injections of ACh gave rise to mechanical responses of the musculature in all patients with myotonia and in two of the six healthy controls as well as in one patient with peripheral neurogenic paralysis of the finger flexors. After large doses (20—25 mg) and occasionally after small doses (1 mg) patients with d.m.

Table IV Pharmacological tests. Neuromuscularly healthy controls

Agent	No. of controls	No. of series	No. of injections	Dose variations (mg)	Mechanical response
Depolarizing agents					
Succinyldicholine	22	25	172	10—100	None
Succinylmonocholine	1	2	9	200—400	None
Decamethonium	1	1	2	2—3	None
Acetylcholine	6	6	54	0 05—20	Two of six controls
Choline	5	5	64	0.05—40	None
Total		39	301		

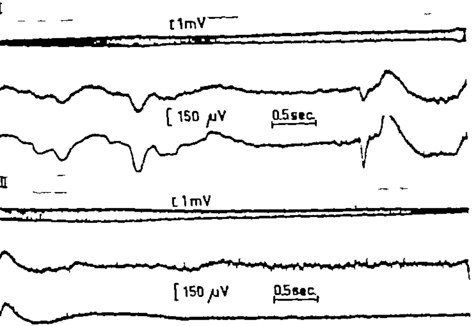
Table V Pharmacological tests Patients with muscular or neuromuscular diseases

Diagnosis	No. of series	Dose variations (mg)	No. of injections	Injections with mechanical response
Succinyldicholine intravenously				
Dystrophia musculorum progressiva	3	10—30	26	0
Amyotrophic lateral sclerosis	2	10	8	3
Arteria spinalis anterior syndrome	1	10	1	0
Peroneal muscular atrophy	1	10	3	0
Paresis of the finger flexors, examined 13 weeks after lesion of right plexus brachialis	2	10—?0	11	8
Same patient examined 20 weeks after the injury	1	10—20	5	5
Total	10		54	16
Acetylcholine or choline (fig in brackets)				
Paralysis of the finger flexors, examined 12 days after lesion of left plexus brachialis	1	0.05—20 (10—80)	7 (8)	5 (0)

With the latter method the isometric registration was combined with simultaneous registration of muscle action potentials from superficial or deep flexors of the forearm and from the hypothenar Ten patients with d.m., two with m.c., four neuromuscularly healthy controls and one patient with paralysis of the finger flexors following a plexus lesion were investigated with the Disa electromyograph. All the experiments, those done by mechanical registration as well as those done with combined registration, are compiled in tables III IV and V For control experiments intravenous injections of the nondepolarizing agents curare and gallamine were given in three patients w th d m. (table III) Additional information concerning the method may be found in papers by Örndahl (43) Örndahl and Stenberg (45) and Petersén, Stenberg and Örndahl (?9

Results

In the experiments performed no essential differences in the effects of the five depolarizing agents on myotonic



. 3 Myotonia congenita, 5 mg succinyldicholine intra-arterially Earlier in the experiment, the ...ent had received 65 mg succinyldicholine intravenously The electrical activity remains during ... regression of the mechanical effect. Mechanical effect about 12 sec.

...ntrols nor in the patient with peripheral neurogenic paralysis.

...rare and gallamine

With curare and gallamine, no mechanical response of the musculature was observed in the patients with d.m. — ...ly a flaccid paresis (block) such as that found in neuromuscularly healthy humans.

...I Simultaneous mechanical and electromyographic registration

In order to analyze the mechanical response more closely experiments with simultaneous mechanical (isometric) and electromyographic registration were carried out. In patients with myotonia an essential difference between the results of highly effective and less effective doses was noted. With highly effective doses (fig 2) a pronounced mechanical effect followed injection of ACh (10 mg) SCh (25—50 mg) and C_1 (5 mg) and in the case of SCh "Geburtshelferstellung" (accoucheur position) of the hand as well. While the mechanical effect was in progress, electrical interference was registered both in the long flexor and in the hypothenar This activity diminished at a more rapid rate than the mechanical effect during regression, and in some instances ceased entirely despite a persisting mechanical effect. This signifies that during regression certain muscle fibres participate in the mechanical effect without simultaneous electrical activity in other words these fibres have probably

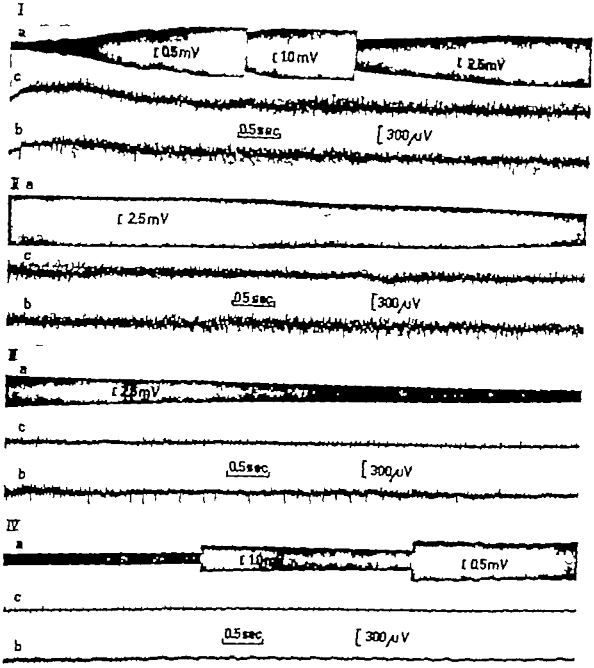


Fig 2 Dystrophia myotonica, simultaneous mechanical and electromyographic registration after 10 mg acetylcholine intra-arterially The tracings are divided into 4 continuous sect ons (I—IV) a Mechanically registered tonus change in the ulnar flexor muscles (mechanical effect) b. Electromyographic registration from the flexor carpi ulnaris muscle. From the hypothenar The electrical activity ceases in spite of a remaining mechanical ffect. The duration of the mechanical effect is about 40 sec.

had a more prolonged muscular effect (45—120 sec) than that reported in the literature for healthy humans (1—15 sec. (11 17))

Choline

Injections of Ch gave rise to mechanical responses of the musculature in all patients with d.m., but not in the healthy

had been detected. This statement was made, however before any investigations with Ch, SCh, SmCh and C_{10} had been carried out.

Myotonic muscle reacted in the same manner as the paralytic and the paretic musculature of two patients with plexus injuries of twelve days and twenty weeks standing when these patients were tested with injections of ACh and SCh respectively A patient with amyotrophic lateral sclerosis of three years duration showed a similar response to SCh, This type of reaction, however did not occur in the severely paretic and atrophic musculature of two patients (arteria spinalis anterior syndrome and peroneal muscular atrophy) who had longstanding injuries of the peripheral motor neuron (13—28 years) Nor was any such reaction observed in the musculature of two patients with primary muscular disease (progressive muscular dystrophy of limb girdle type)

Myotonic human muscle, when tested with depolarizing agents, showed a reaction similar to that previously observed in certain animal muscles, i.e. both healthy and denervated frog and bird muscles, muscles from mammalian foetuses and from newborn mammals, denervated cat muscle and botulinum-intoxicated cat muscle (20)

Analysis of the mechanical response recorded by simultaneous mechanical and electromyographic registration showed that highly effective doses of ACh, SCh and C_{10} caused contraction, and probably contracture as well, in the myotonic muscle. The same behaviour was observed in peripheral paralytic human muscles after ACh.

As to the assumption that contracture occurred it might be argued that the registration of the mechanical effect may reflect several muscles working together or in opposition to each other and that it is impossible to determine the role of each individual muscle. The work of the muscles responsible for features of like magnitude on the tracing during progression and regression is not necessarily equal. During the progression, when there was interfering activity in the flexors, the effectivity of the antagonists may have been greater than during the regression of the mechanical effect when interference was no longer registered It is also conceivable that, when electrical activity was absent, the muscle from which the electrical registration was made no longer participated in the mechanical effect, the latter being brought about by synergists of the investigated muscle. These sources of error can be eliminated only by studies on isolated muscle preparations, and such studies are impracticable in living humans.

Investigations of this type have however been made on isolated frog and bird muscles and on denervated mammalian muscle (4 5 32, 42) These studies involved simultaneous mechanical and electromyographic registration, and the tracings obtained conformed essentially with those recorded during the present investigations on myotonic and peripheral paralytic human muscle. Since contracture is known to occur during experiments on these animal muscles, it is highly probable that such a contracture also occurred in the investigated ulnar flexor muscles of the present myotonic and peripheral paralytic patients.

In further support of the assumption that contracture takes place, it may be pointed out that when the dose of a depolarizing agent was increased in a test series, the duration of the electrical activity was not prolonged commensura-

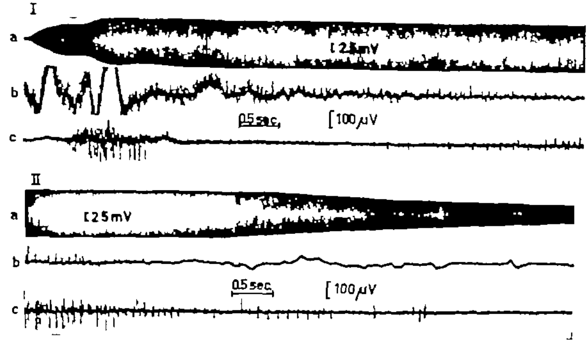


Fig 4 Paralytic human musculature after plexus lesion. 20 mg acetylcholine intra-arterially Results as in fig 2 Mechanical effect about 18 sec.

undergone contracture. After less effective doses (fig 3) of ACh (0 5 mg) and SCh (5 mg) however the electrical activity remained during the entire regression of the mechanical effect a circumstance which indicated contraction of the musculature. In the long flexor however during the mechanical effect only a few action potentials appeared after ACh, probably because of insufficient distribution of the agent to this muscle.

In the neuromuscularly healthy controls no mechanical effect was observed to follow injections of ACh Ch SCh or C_{10}. After half the total number of ACh injections, however electrical activity was registered in the form of a few action potentials per second but no interference was noted The same result followed an injection of SCh. No electrical activity was, however observed to follow injections of Ch and C_{10} except for one Ch injection (5 mg) where a few potentials were noted.

After injection of ACh (20 mg) the tracing from the patient with arm paralysis following a plexus injury (fig 4) indicated a contracture similar to that found in myotonia cases. Injections of Ch however elicited no mechanical effect only an interfering activity was registered.

(Comprehensive reports of the results have been published by Örndahl (43) Örndahl & Stenberg (45) and Petersén, Stenberg & Örndahl (29))

Discussion

These investigations show that the response of myotonic muscle to Ch SCh SmCh and C_1 differs from that of healthy human muscle.

Pipberger (30) however who made a summary of investigations on myotonic muscle, pointed out that in pharmacological tests no difference between myotonic muscle and healthy human muscle

have demonstrated that multiple end plates may be present on the same muscle fibre during re-innervation in humans. If the presence of multiple end plates is a pre-requisite for myotonia, an active myotonic phenomenon should be manifest during re-innervation of the musculature. This is indeed the case (12, 22). However no investigations have been made to ascertain whether multiple end plates and myotonia co-exist in the same muscles after re-innervation. This myotonia acquisita has been attributed to infection, intoxication, metabolic disturbances and trauma. Krabbe (22) emphasized that this form of myotonia may be caused by a previous neuritis. Frykholm (12) compiled thirty cases with cervical nerve root compression. In three of them myotonia-like symptoms were registered from the hand in the form of an active myotonic phenomenon. The series presented here included a patient with arm paresis resulting from plexus injury and investigated twenty weeks after the injury when the muscle strength was almost restored. The patient's response to SCh in the healthy arm was normal but the reaction to SCh in the paretic arm corresponded to that of a patient with myotonia. An investigation of the presence of multiple end plates provided no data owing to a technical mishap.

No reports have been published concerning the presence of multiple end plates in cases of arteria spinalis anterior syndrome. The musculature in cases of amyotrophic lateral sclerosis and peroneal muscular atrophy has been the subject of histological investigations (8). These investigations have shown incipient re-innervation as well as degeneration of the newly formed nerve branches and the end plates. The presence of multiple end plates was not reported, however.

In cases of dystrophia musculorum progressiva the innervation is not initially affected (8) and the literature on this disease contains no report of the presence of multiple end plates in the still intact musculature. Thus, the anatomical prerequisite for contracture has not been demonstrated in this disease. The only sign of contracture observed in the controls studied here was in a patient with amyotrophic lateral sclerosis whose reaction suggested a mechanical response.

Some authors have compared the myotonic phenomenon with neuromuscular contracture and have thereby elucidated the effect of endogenous ACh in patients with myotonia. Bremer (3) made a statement that "myotonic rigidity" of human pathology presented striking analogies in properties and mechanism with neuromuscular contracture in frogs. He also drew the following conclusion: "The myotonic rigidity is a neuromuscular contracture of abnormal muscles, provoked by a summation of normal nerve impulses." Gasser (13) likewise drew an analogy between the contracture produced by indirect stimulation (neuromuscular contracture) and the behaviour of muscles in Thomsen's disease. Ravin (31) wrote: "It is evident that myotonia following voluntary contraction is comparable to a neuromuscular contracture."

By high-frequency nervous stimulation of the musculature (40—50 per second) which releases endogenous ACh, it has been shown that the relaxation time for multiple-innervated frog muscle fibres is 50 to 100 times longer than that for focally innervated muscles of the same animal (23). The relaxation of myotonic skeletal muscle after vigorous voluntary movements does not occur in a fraction of a second as in healthy human muscle,

tely with that of the mechanical effect. *In many instances the electrical* activity showed an early diminution or ceased entirely despite a remaining mechanical effect

A pre-requisite for the occurrence of contracture, according to *Thesleff* who carried out experiments on animals, is that the depolarizing agents are able to act upon an increased receptor surface (personal communication) A close relationship between the area of the receptor surface and the intensity of the ACh-induced contracture has been observed (10)

In muscle fibres from mammalian foetuses and from newborn mammals (9) as well as in denervated frog muscle (27 28) cat muscle (1) and in botulinum intoxicated cat muscle (38) the ACh-sensitive surface is enlarged This surface is not limited to the end plate region but involves most, or all of the muscle fibre membrane (39) As has been pointed out above, these muscles show both contraction and contracture in response to depolarizing agents.

It has previously been shown that the muscle fibres in certain frog and bird muscles are equipped with multiple and sometimes closely situated end plates while other fibres contain only one plate (14, 16 18) Ginsborg (15) states that frog and bird muscle fibres with multiple end plates respond to prolonged depolarization with contracture — a response which is not observed in those animals whose fibres are innervated with a solitary end plate.

Wilkinson (41) who reported histological investigations of normal musculature in cats found the muscle fibres to possess only one end plate. He pointed out however that other authors had observed fibres with two end plates. Healthy adult cat muscle showed no contracture in response to the agents used

No physiological determinations of the ACh sensitive surface of myotonic or denervated muscle fibre in humans have been published.

The state of the end plates in normal and pathological human musculature has been studied (8) In six patients with d.m. the muscle fibres were found to possess multiple and usually enlarged end plates of normal appearance This finding was based on histological or histo-chemical investigations of biopsies from e. g., the flexor muscles of the forearm. Thus, the flexors of the forearm probably contain the anatomical pre-requisite for a contractural response to ACh and other depolarizing agents. MacDermot (25) observed multiple end plates in the deltoid muscle of patients with d.m. Multiple enlarged end plates were also found in m.c. patients (personal communication from Woolf)

The skeletal muscle fibre of neuromuscularly healthy humans contains only one end plate (8) Double end plates have, however been observed in a small number of cases (2.3 %) (8) Only in vocal cord muscle has the majority of the fibres been shown to possess multiple end plates (33) In this investigation normal human skeletal muscle did not show a contractural response to the depolarizing agents used. To summarize the presence of multiple end plates and the enlargement of the receptor surface probably constitute an anatomical pre requisite for the contractural response of patients with myotonia to injection of ACh and other depolarizing agents.

The status during re-innervation provides further evidence of the importance of multiple end plates. Coërs & Woolf (8)

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but is prolonged for several seconds up to 1/2 min. (19)

It is known that endogenous and exogenous ACh act in a similar way on the end plate (7) The results of the present experiments with exogenous ACh could therefore be applicable to endogenous ACh The active myotonic phenomenon could then be explained as a contracture caused by the influence of endogenous ACh on muscle fibres with multiple end plates and enlarged receptor surfaces.

This view as to the cause of the active myotonic phenomenon has not, as far as I have been able to ascertain, been previously advanced in the medical literature.

Summary

In eighteen patients with dystrophia myotonica and in three patients with myotonia congenita, the myotonic ulnar flexor muscles were studied by stimulation with succinyldicholine (201 injections) succinylmonocholine (17 injections) decamethonium (20 injections) acetylcholine (230 injections) and choline (73 injections) The controls comprised twenty eight patients (301 injections) without and seven patients (54 injections) with muscular or neuromuscular diseases. The mode of recording was either isotonic or isometric, the latter mode being combined as a rule, with electromyographic registration.

1 After injection of the above agents a mechanical response of the musculature was registered isotonically and isometrically in all patients with myotonia. In the control cases without muscular or neuromuscular diseases only acetylcholine elicited a mechanical response. The paretic musculature of a patient with amyotrophic lateral sclerosis and the paretic and paralytic musculature, respectively of two patients with plexus injuries showed a mechanical response to injection of succinyldicholine or acetylcholine On the other hand no mechanical response was recorded in the severely atrophic musculature of two patients with arteria spinalis anterior syndrome and peroneal muscular atrophy respectively Nor was any mechanical response registered in two patients with progressive muscular dystrophy of limb girdle type.

2 The nature of the mechanical response elicited by succinyldicholine, decamethonium and acetylcholine was analyzed In patients with myotonia the *electrical activity was found to diminish* more rapidly than the mechanical effect and, in some instances, to disappear completely even though the mechanical effect persisted — a situation which suggested that contraction as well as contracture occurred in a large number of muscle fibres. Similar observations were made after intra arterial injections of acetylcholine in the paralytic arm of a patient with a plexus lesion.

3 Myotonic muscle probably responded to the relevant agents with a contracture similar to that previously observed in certain frog and bird muscles, and like those possesses multiple end plates on *each muscle fibre. It is possible that the* active myotonic phenomenon constitutes a contracture arising from the effect of endogenous acetylcholine on muscle fibres with multiple end plates.

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From King Gustaf V:s Research Institute, Stockholm, Sweden

Amino Acid Analyses of the Rheumatoid Factor Macroglobulin

Preliminary Report

By

N. Svartz, J. Liljamaa and B. Ericson

The studies here reported were made in order to discover whether the characteristics of the macroglobulin-containing rheumatoid factor (RF) might be further elucidated by amino acid analyses. The results were partially presented at the Swiss Academy of Medical Sciences in December 1961. Our aim in this series of experiments was to isolate the rheumatoid factor by the most careful possible means so that it might be in unchanged condition when actually analyzed. At every stage in the purification of RF checks were made to ascertain that its hemagglutinating activity was unimpaired.

Material and methods

Fresh serum from blood drawn on an empty stomach under sterile conditions was used as starting material. As soon as sufficient quantity of serum had settled out, it was inactivated and the heterophil agglutinins were removed by absorption with fresh red cells. Thereafter one part serum (usually 6 ml) was diluted with 14 parts aq. dest. The mixture was left for 48 hours at + 4° C, followed by centrifugation and removal of the supernatant. The precipitat was dissolved in saline or phosphate buffer to the same volume as the original serum.

Chromatography was then performed by the method of Peterson and Sober using the cation exchanger carboxymethyl cellulose. Phosphate buffer was used for equilibration of the column (see below). The redissolved cold precipitate was diluted with an equal volume of phosphate buffer (the same as for equilibration). 10 ml of this mixture was poured on to the column and 20 ml of equilibrating buffer was then added. After about 1 hour a gradient was added (see below), 100 ml of gradient buffer A being admitted to the lower tube and 100 ml of buffer B to the upper. About 40 fractions were collected, each containing about 4 ml.

Buffer solution for equilibration. Equal parts of 0.9 % NaCl and 0.05 M NaH_2PO_4.

Gradient buffer A. Equal parts of phosphate buffer and 0.9 % NaCl. Phosphate buffer: 410 ml of 0.2 M NaH_2PO_4 + 90 ml of 0.2 M Na_2HPO_4.

Gradient buffer B. Equal parts of phosphate buffer and 0.9 % NaCl. Phosphate buffer: 320 ml of 0.2 M NaH_2PO_4 + 60 ml of 0.2 M Na_2HPO_4.

The hemagglutination test was performed on the fractions containing most protein. A part of the fraction showing the strongest hemagglutination was taken for ultracentrifugation. If ultracentrifugation revealed a single, isolated, completely homogeneous and symmetrical peak, the fraction was subjected to amino acid analysis. To obtain sufficient material, fractionation usually had to be done on the cold precipitate from 6—12 ml of serum.

After dialysis and hydrolysis of the fraction, the amino acids were determined by the method of Moore and Stein. The analyses were made by J. Liljemaa at Professor E. Jorpes' Chemical Institute.

Results

Table I shows in tabular form four separate attempts to analyse the amino acids in isolated RF macroglobulin. The agreement between the

Submitted for publication August 31, 1962.

SUBJECT INDEX

Anodynes

Arteries

Asthma

Blood

Table I Amino acid analyses

Amino acids	RF Macroglobulin				Macroglob. Blood donor	γ-globulin 7 S
	A—L I	A—L II	E son	B son		
Alanine	4.2 (%)	4.7 (%)	4.1 (%)	5.4 (%)	5.0 (%)	3.6 (%)
Arginine	4.0	3.5	4.8	4.4	6.3	4.0
Aspartic acid	8.9	9.3	8.4	8.8	8.5	8.5
$CySO_3H$	3.0	1.8	2.5	2.7	3.2	1.5
Glutamic acid	11.7	12.8	10.9	12.6	11.8	12.1
Glycerin	3.1	3.2	3.7	3.3	2.4	4.0
Histidine	2.0	1.8	2.7	2.5	3.8	2.2
Isoleucine	2.9	3.0	3.0	2.9	1.9	2.2
Leucine	8.0	8.7	7.6	8.5	8.0	7.6
Lysine	5.3	4.9	6.1	6.0	10.5	7.6
Methionine + met. sulfox.	0.5	0.6	0.7	0.5	0.5	0.6
Phenylalanine	5.1	4.7	4.3	5.6	4.9	4.3
Proline	5.1	4.7	6.6	4.9	3.7	7.8
Serine	7.4	7.2	7.5	6.3	5.0	10.4
Threonine	7.4	7.5	6.8	6.4	5.1	7.8
Tyrosine	3.1	3.4	3.7	3.7	2.6	3.4
Valine	9.2	9.3	7.8	6.6	8.3	8.5
$Amide-NH_3$	—	—	—	—	—	1.5

analyses is striking. It was naturally desirable to compare the results with the content of amino acids in purified macroglobulin from a healthy person. As repeatedly pointed out by us previously the macroglobulin from healthy persons is hardly precipitated in the cold. To get a comparative material treated in the same way as rheumatoid serum, we performed cold fractionation with normal serum (blood donor) on approximately 10 times the scale used in the examination of rheumatoid serum. This gave us the quantity required for the experiment. The subsequent purification and the amino acid analyses were also done on the same lines as for RF. A representative analysis is given in column 5 of table I. The important finding was made that the normal macroglobulin had essentially the same quantitative composition as the RF macroglobulin.

In this context it is important to emphasize that the normal macroglobulin, even if concentrated, does not give rise to a positive hemagglutination reaction. As for untreated normal serum, such serum, if concentrated, may sometimes give an insignificant hemagglutination titer, i. e. up to 1 : 32. But in our modification of the Waaler Rose test we have reckoned agglutination up to 1 : 32 as a negative reaction. Our experience is based on about 22,000 hemagglutination reactions. Although the RF macroglobulin and the normal macroglobulin have roughly the same amino acid composition, the former gives rise to hemagglutination, the latter not at all or only to a very slight extent.

Since some authors consider that the macroglobulins can be split into 6—7 S globulins, it was desirable to analyse isolated 6—7 S globulin. This we obtained from the firm of Kabi. As will be seen from column 6 of table I there are certain distinct differences between the macroglobulins and the 6—7 S globulin especially in the percentage of serine, tyrosine and proline, which was relatively lower in the macroglobulin, and of alanine, which showed a somewhat higher percentage. The basic amino acids showed roughly the same distribution in all cases.

Summary

A report is given of amino acid analyses on isolated macroglobulin containing rheumatoid factor. A comparison with purified macroglobulin from blood donors shows that the difference between the macroglobulins does not lie in their content of amino acids.

Since the carbohydrate content is about the same, the difference in properties of the macroglobulins is in all probability due to their dissimilar molecular structure.

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Collagen diseases

Diabetes mellitus

Brain

Circulation

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Diabetes mellitus

Endocrine glands

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Hypertension

Infectious diseases

Intestines

Kidney

Liver

Lung

Metabolism

Muscles

Poisoning

Roentgenography

Skin

Stomach

Liver

Lung

Metabolism

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